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Studies in
The Aetiology of Epidemic Influenza

BY

JAMES McINTOSH, M.D.

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INTRODUCTION

THE present Report gives the results of studies undertaken by Professor McIntosh during the epidemic of influenza in the autumn and winter of 1918–19 and later. The completion of some of the experimental work and some other circumstances have delayed the publication of the Report, and it should be understood that it was completed in its present form in 1920. The Council believe that the inclusion of the Report in this Series may be convenient to many other workers in the subject.

Visitations of influenza on a pandemic scale occur at relatively long intervals of time, and it is a matter of great importance that they should be studied from every point of view. The investigation of the true cause of the disease can, indeed, be pursued effectively only at such times, because the clinical conception of the disease 'influenza' is not defined with enough precision to allow us to say whether the minor inter-pandemic outbreaks or the innumerable sporadic cases of a similar affection are really instances of one and the same disease.

The Council have previously published other Reports upon work done during the 1918–19 pandemic, and they hope shortly to publish the results of more recent studies made within the past year. The results of the careful researches upon which Professor McIntosh now makes a full report—in supplement to an earlier preliminary communication—have been to lead him to the opinion that Pfeiffer's bacillus is the true cause of epidemic influenza, and he has marshalled the evidence in favour of this view with much force and skill. This is a view which, as is well known, was not held by all the writers in the previous Report published by the Council on the subject, and it may be well to take this opportunity of repeating that the Medical Research Council does not hold itself responsible for the scientific conclusions reached by the authors of reports which they may publish. The object of the Council

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is to facilitate publication of the results of scientific work, leaving the evidence and the arguments offered by the authors to bring to other students appropriate conviction as to the justice of the conclusions reached. It is not likely that in this difficult subject, any more than in other directions of scientific progress, truth will be finally reached except through the clash of conflicting opinions.

Medical Research Council,
15 York Buildings,
Adelphi,
London, W.C. 2.

27 *May* 1922.

STUDIES IN THE AETIOLOGY OF EPIDEMIC INFLUENZA.[1]

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1. Introduction.

These researches were undertaken with the idea of throwing some light on the aetiological agent of acute influenza and were mainly carried out during the recrudescence of the epidemic in the autumn and winter of 1918–19. Unfortunately at this period it was not possible to give as much attention to the problem as was necessary owing to other more urgent claims on one's time.

In order to make clear the line of argument pursued in this work, it is thought necessary to summarize here the conditions which must be fulfilled before a virus can be presumed as the cause of a disease. These are stated in the so-called Postulates of Koch. Koch did not actually tabulate any definite laws, but indicated the principles upon which the virus of an infective disease can be accepted as the cause. Briefly Koch's three main principles are as follows:

(1) The virus should be capable of being recognized in a large proportion of the cases of the disease, and preferably in relation to the chief lesions.

[1] Awarded the Liddle Triennial Prize, 1920. London Hospital Medical College, University of London.

(2) The virus should be living, i.e. should possess the power of indefinite propagation under suitable conditions. This can be demonstrated in two ways—(*a*) by passage through a series of animals; (*b*) by a similar passage on artificial media.
(3) The virus should be capable of reproducing the disease in animals.

The third is the final criterion by which a virus may be adjudged the cause of a disease. The term virus is used in a wide sense and must include the effects of any toxin produced by the virus. Thus, typical tetanus can be produced by the injection of a filtered culture.

In the application of the above tests to certain infections, and in particular in the case of Influenza, many difficulties arise. It may be pointed out that it is impossible to communicate certain human diseases to laboratory animals. In others a disease is produced but it does not show the typical lesions as seen in man, while the clinical phenomena of disease in animals cannot be judged with any degree of success.

In the case of influenza an additional difficulty arises from the fact that influenza up to the present has been a *clinical concept*, the diagnosis resting mainly on the presence of a catarrhal disorder of the respiratory tract which tends to assume epidemic and pandemic characters. Clinical methods are unable to distinguish between epidemic catarrhs due to entirely different microbes.

When the outbreak first appeared in the spring of 1918 doubt was expressed in many quarters as to its being true influenza. This was particularly the case amongst our troops in France, where the disease was given the name of P.U.O. (Pyrexia of Unknown Origin). But after a more careful survey of the symptoms and pathological lesions, the opinion gradually was formed that the epidemic was one of true influenza.

It is outside the scope of this investigation to go into the clinical features of the epidemic. But it is easy to see that the clinical features as described by Wirgman (1), Connor (2), and others are very similar to, if not identical with, those observed during previous epidemics (Thompson) (3).

The epidemic at its onset seems to have taken by surprise the bacteriologists as well as the clinicians, for the researches at this period are few and imperfect. The exact place and date when the epidemic first appeared is very indefinite. The Spanish origin of the outbreak is doubtful, although a large epidemic did occur in that country in the early spring of 1918. But an examination of the literature shows that local epidemics of apparently typical influenza were occurring in the British Army at home and in France even earlier than this, i.e. during the winter of 1916–17. Hammond, Rolland, and Shore (4), and Abrahams, Hallows, Eyre, and French (5).

The first large epidemic in the British Army occurred in May, June, and July, 1918, and then it was of a mild type. The symptoms lasted only a few days, and in many instances these were so

indefinite that the condition came to be known as 'three-day fever'. In all, some 226,615 cases were reported. In consideration of this and the relatively small amount of bacteriological work done it might be as well not to attach much importance to the findings at this stage of the epidemic.

In the British Army the disease recurred in November and December, 1918, when some 93,670 individuals were incapacitated from duty. The deaths are given as 5,555, which corresponds to 6·2 per cent. (7, 9).

In the Navy there was a severe outbreak in the Grand Fleet in May, when about one-ninth of the total strength was affected. Amongst the civil population the epidemic did not appear in any strength until June and July, although localized outbreaks were noted in May. In November and December there was a severe recrudescence of the epidemic with a high mortality (8). In other European countries very similar histories can be obtained.

2. Bacteriological Investigations.

The material examined was obtained from all types of influenza, and special care was taken that as little delay as possible should elapse between the collection and examination. The routine practice was to first make a film of exudate (sputum, pus, or lung juice), stain with Gram and counterstain with weak fuchsin or neutral red. By this means valuable information was obtained as to which type of bacteria predominated. Then, for the isolation of the various bacteria three different media were employed, namely, Matthews' trypsinized blood agar, serum agar, and ordinary agar (6). The first medium was used for the isolation of *B. influenzae*, for which it exhibits considerable selective powers. At the same time it inhibits many of the other bacteria which usually tend to overgrow the more delicate *B. influenzae*. It is unfortunate, however, that this medium is not always constant in these two qualities. The serum agar and ordinary agar provided a means of isolating the various Gram-negative and Gram-positive cocci present. A sufficient degree of dilution can be obtained with three tubes of each medium; one platinum loopful of the sputum or pus is rubbed over the three tubes in series, and the tubes then incubated over night. Occasionally the pus was emulsified in a few cubic centimetres of saline, and one drop of the fluid used to inoculate the tubes or plates in series.

For the preservation of the various strains of *B. influenzae* I have found boiled blood agar most suitable. This medium is easily prepared by adding 5 c.cm. of defibrinated blood (human, sheep, or rabbit) to 100 c.cm. of ordinary agar which has been melted and cooled to about 48° C., and then placing the flask in a water bath and gradually raising it to boiling-point. The flask is then removed after two or three minutes and the medium distributed into tubes or plates as desired. It is unnecessary to filter off the coagulum, which is a troublesome procedure. *B. influenzae* grows luxuriantly on this medium, and will remain alive without being subcultured

for at least ten days, which is a matter of some importance when a number of strains are being studied.

Incidence of the various Micro-organisms.

As bacteria, in particular cocci, are present in any catarrhal condition, or for that matter in the healthy respiratory passages, a mere statement of their presence or absence would not be of much value. Thus, unless a particular organism is present in considerable numbers as indicated by a predominance of that type in the films and cultures no attention was paid to it. In many instances the film diagnosis gave a better index of the predominating type than did the cultures, which grew a great variety of organisms. This was not, of course, the case with regard to *B. influenzae*, as that organism is small and does not stain well, so that, unless present in considerable numbers, it is likely to be missed. The value of a selective medium like Matthews' is that it prevents the overgrowth of the more delicate *B. influenzae* by the other organisms. In addition the film preparations of the original pathological exudate present a fairly reliable means of differentiating between pneumococci and streptococci. In fact, in my hands I have found the morphological differentiation of pneumococci to be quite as good as many of the cultural tests now advocated.

The pathogenic microbes most commonly found in these influenza cases, either alone or in association with *B. influenzae*, were streptococci, pneumococci, staphylococci, and Gram-negative cocci meningococci, *Micrococcus catarrhalis*, and *M. pharyngis siccus*. It would appear that with the exception of *B. influenzae* the associated microbes tended to go in waves, as at one period one type predominated and at another another. Gram-negative cocci were more frequent in the earlier investigations and pneumococci towards the end of the epidemic.

An analysis of two groups of cases (see tables Nos. II, III) examined gave the following:

1. *Sputum from Acute Influenzal Broncho-Pneumonias.*

		Predominating Organisms.			
No. of cases.	*B. influenzae.*	*Pneumo-cocci.*	*Strepto-cocci.*	*Staphylo-cocci.*	*Gram-negative cocci.*
70	58	22	24	19	23
Per cent.	82·8	31·4	34·2	27·1	32·8

2. *Bronchial pus, lung exudates, &c., from series of post-mortem broncho-pneumonias examined during the epidemic period Oct. 1918 to April 1919.*

		Predominating Organisms.			
No. of cases.	*B. influenzae.*	*Pneumo-cocci.*	*Strepto-cocci.*	*Staphylo-cocci.*	*Gram-negative cocci.*
68 including 8 controls.	40	20	22	21	19
Per cent.	58·8	29·4	32·3	30·8	28·9

But when all controls and those cases where no signs were found definitely characteristic of the epidemic are excluded [1] the following figures are obtained:

No. of cases.	*B. influenzae.*	*Pneumo-cocci.*	*Strepto-cocci.*	*Staphylo-cocci.*	*Gram-negative cocci.*
48	36	13	16	10	13
Per cent.	75·0	27·0	33·3	22·2	27·0

A complete differentiation of the streptococci and pneumococci was not possible in this research, and distinction between the two was based on morphological appearances in films, on the character of the colony on serum agar and on certain fermentation reactions. Of these the fermentation of inulin was perhaps the most reliable. In a small series, in which most of the suggested cultural tests were employed, results were obtained which would tend to indicate that at least three types of pneumococci and two of streptococci were present. But further investigation was necessary to enable a quite definite statement to be made.

Incidence of B. Influenzae. *In the epidemic period.*

B. influenzae proved to be the most frequent pathogenic microbe present in the various pathological secretions of acute influenza. The relative frequency in the various types of cases and lesions as shown in Tables Nos. I, II, and III, is given below in tabulated form.

(a) *Early acute uncomplicated influenza* (posterior naso-pharyngeal swabbing).
No. examined 17; *B. influenzae* present in 13 = 76·4 per cent.

(b) *Broncho-pneumonic types.* (Sputum.)
No. examined 70; *B. influenzae* present in 58 = 82·8 per cent.

Post-mortem material.
No. examined 48; *B. influenzae* present in 36 = 75·0 per cent.

Special series which included tracheal examination, naso-pharynx, &c.
No. examined 16; *B. influenzae* present in 15 = 94 per cent.

Blood-cultures as a rule were sterile; on a few occasions streptococci were found shortly before death.

Empyemata, 12 examined; *B. influenzae* found twice.

The close agreement between these groups is very striking, and is highly suggestive as to the aetiological importance of *B. influenzae.* Not only did this organism predominate, but in a large number of cases it was present in such numbers that pure growths, apart from a few other colonies, were obtained with great facility.

[1] Nos. 5, 10, 16, 18, 20, 22, 23, 27, 32, 34, 35, 36, 38, 46, 51, 55, 56, 62, 64, 65 (Table III) did not present a typical influenzal broncho-pneumonia, while nos. 5, 32, 35, 36, 46, 51, 62, and 65 had been selected as controls.

Table I.

Pharyngeal Examinations of Acute Uncomplicated Influenza Cases.

(Nurses epidemic, 28th Oct. 1918.)

1.	Q.	Temp. 102° F.	Cultures gave only a few typical *B. influenzae* colonies.
2.	C.	Temp. 103° F.	No *B. influenzae* seen or cultivated—cultures showed a stout Gram-negative bipolar bacillus.
3.	K.	Temp. 102·2° F.	No *B. influenzae*, very few bacteria, only a few of the bipolar bacilli.
4.	T.	Temp. 100° F.	*B. influenzae* isolated, also a Gram-negative coccus (*M. catarrhalis*) and a stout bipolar bacillus.
5.	S.	Temp. 101° F.	*B. influenzae* isolated.
6.	S.	Temp. 101·2° F.	*B. influenzae* present, also Gram-negative cocci and a bipolar bacillus.
7.	G.	Temp. ?	*B. influenzae* present.
8.	M.	Temp. 103° F.	Numerous *B. influenzae* colonies with a few *Staphylococcus albus* colonies.
9.	C.	Temp. 102·5° F.	No *B. influenzae* isolated.
10.	B.	Convalescent case	*B. influenzae* present in considerable numbers.
11.	B.	Temp 101·5° F.	*B. influenzae*, also Gram-negative coccus present.
12.	I.	Temp. 101° F.	No *B. influenzae* found.

Table II.

Sputum of Acute Influenzal Broncho-pneumonias examined during the Epidemic Period Oct. 1918–June 1919.

No.	*Initials.*	*Age.*				*B. influenzae.*	*Pneumococci.*	*Streptococci.*	*Staphylococci.*	*Gram-negative cocci.*	*Remarks.*
1.	F. B.	29	...	...	...	+	...	...	...	+	
2.	S. N.	18	...	...	...	+	+	...	...	+	
3.	C. S.	20	...	...	...	+	...	...	+	+	
4	R. G.	40	...	...	...	+	...	...	...	...	Practically a pure culture.
5.	E. P.	28	...	...	...	—	+	...	...	...	
6.	G. G.	27	...	...	...	+	...	+	+	...	Pleuritic fluid contained only streptococci.
7.	P.	26	...	...	...	+	...	...	+	...	
8.	W.	40	...	...	...	+	...	...	+	...	Also *B. Friedländer*.
9.	B. H.	50	...	...	...	+	+	...	...	...	
10.	R.	45	...	...	...	+	...	...	...	+	
11.	E. H.	?	...	...	...	+	+	+	...	...	
12.	B. R.	?	...	...	...	+	+	...	...	...	
13.	W. P.	30	...	...	...	—	...	+	+	+	
14.	H. W.	17	...	...	...	+	...	...	+	+	
15.	D. M.	24	...	...	...	—	...	+	+	+	
16.	E. H.	?	...	...	...	+	+	...	+	...	
17.	C. G.	24	...	...	...	+	+	...	...	+	
18	W. C.	24	...	...	...	+	+	...	...	+	
19.	P. S.	25	...	...	...	+	+	...	...	+	
20.	Dr. X.	?	Broncho-pneumonia			+	...	+	+	...	
21.	W. G.	32	...	...	...	+	...	+	...	+	
22.	M. K.	36	...	...	...	+	...	...	+	+	

No.	*Initials.*	*Age.*				*B. influenzae.*	*Pneumococci.*	*Streptococci.*	*Staphylococci.*	*Gram-negative cocci.*	*Remarks.*
23.	M. G.	31	...	...	...	—	...	+	+	...	
24.	R. G.	23	...	...	...	+	...	...	...	...	Almost a pure culture obtained.
25.	A. D.	26	...	...	...	+	—	...	...	...	
26.	J. M.	42	...	...	...	+	...	...	+	...	
27.	M. S.	23	...	...	...	—	...	+	...	+	Microscopically positive, but medium overgrown with *B. proteus.*
28.	S. H.	45	...	...	...	—	+	+	...	...	
29.	L. A.	11	...	...	...	+	+	+	...	+	
30.	B. W.	32	...	...	...	+	+	...	...	...	Cultures on Matthews' medium gave almost pure *B. influenzae.*
31.	D. K.	25	...	...	...	+	...	...	+	...	
32.	H. F.	38	...	...	...	—	...	+	...	...	
33.	W. P.	45	...	...	...	+	...	...	...	+	
34.	F. R.	39	...	...	...	+	...	+	...	...	
35.	C. B.	25	...	...	...	+	...	...	+	...	
36.	S. G.	51	...	...	...	+	...	+	...	...	
37.	S. H.	26	...	...	...	—	+	+	...	+	
38.	J. M.	54	...	...	...	+	...	...	...	...	Culture almost pure.
39.	H. G.	40	...	...	...	+	...	+	...	...	Pleomorphic streptococcus.
40.	F. M.	35	...	...	...	+	...	...	...	+	
41.	R. H.	39	...	...	...	+	...	...	...	...	Culture almost pure.
42.	M. M.	32	...	...	...	—	+	...	...	...	
43.	H. G.	9	...	...	..	—	...	...	+	...	Sample of sputum appeared to be saliva only.
44	T. G.	19	...	...	...	+	...	...	...	...	
45.	K. M.	31	..	...	...	+	...	+	...	...	
46.	I. B.	22	...	...	...	+	...	+	...	...	
47.	E. C.	17	...	...	...	+	+	...	...	+	
48.	W. B.	17	...	...	...	+	...	...	...	+	
49.	S. I.	31	...	...	...	+	+	+	...	...	
50.	T. B.	?	...	...	...	—	+	...	...	...	
51.	S. C.	29	...	...	...	+	...	+	+	...	
52.	A. G.	27	...	...	...	+	...	...	...	...	T.B. present.
53.	S. W.	41	...	...	...	+	...	...	...	...	
54.	G. P.	27?	...	...	...	+	...	...	...	+	
55.	E. L.	?	...	...	...	+	+	...	...	...	
56.	D. C.	30	...	...	...	+	+	...	+	...	
57.	A. G.	32	...	...	...	+	...	...	...	...	
58.	I. P.	16	...	...	...	+	...	+	...	...	
59.	M. J.	28	...	...	...	—	...	+	...	...	
60.	K. Q.	26	...	...	...	+	...	+	+	...	
61.	W. M.	37	...	...	...	+	...	...	...	+	
62.	M. S.	28	...	...	...	+	+	+	...	...	
63.	I. S.	28	...	...	...	+	+	...	...	...	
64.	C. C.	22	...	...	...	+	+	...	...	...	
65.	F. H.	24	...	...	...	+	...	...	+	+	
66	B. S.	20	...	...	...	+	...	...	...	...	Almost pure culture, T.B. present.
67.	B. H.	50	...	...	...	+	...	...	...	...	
68.	C. N.	38	...	...	...	+	...	...	...	...	
69.	W. R.	36	...	...	...	+	...	...	...	...	
70.	J. H.	22	...	...	...	+	...	+	...	+	

Table III.

Series of Broncho-pneumonias with Controls examined at autopsies, during the Epidemic Period Oct. 1918–June 1919.

The lesions printed in italics were macroscopically suggestive of acute influenza.

Case.	*Post-mortem No.*	*Age.*	*Macroscopic diagnoses.*	*B. influenzae.*	*Pneumococci.*	*Streptococci.*	*Staphylococci.*	*Gram-negative cocci.*
1	455	22	Septicaemia, bronchopneumonia, brown induration of lungs. Chronic rheumatic endocarditis. Recent miscarriage.	+	+	+	..	...
2	458	19	*Red consolidation of lungs and purulent bronchitis.*	+	...	+	...	...
3	462	29	*Subacute, purulent, bronchiectasis and organizing bronchopneumonia.*	+	...	...	...	+
4	463	23	*Bronchopneumonia*, chronic rheumatic endocarditis.	+	+	+	...	+
5	464	27	Slight pneumonia in back pressure lungs, chronic rheumatic endocarditis.	−	+	+	...	...
6	465	22	*Bronchopneumonia.*	−	...	+	...	...
7	474	2	*Bronchopneumonia.*	−	...	...	...	+
8	475	3	*Bronchopneumonia.*	+	...	...	+	+
9	472	27	*Bronchopneumonia*, acute and chronic rheumatic endocarditis.	+	...	...	...	...
10	467	9	Bronchopneumonia.	−	...	...	+	...
11	470	44	*Haemorrhagic, purulent bronchopneumonia.*	+	...	...	+	+
12	487	20	*Haemorrhagic bronchopneumonia.*	+	...	...	...	+
13	492	25	*Empyema: bronchopneumonia with abscesses.*	−	...	...	+	+
14	498	34	*Red pneumonic consolidation of lungs.*	+	+	...	...	...
15	494	40	*Purulent bronchopneumonia, fibrino-purulent bronchitis.*	+	...	+	...	...
16	502	28	Membraneous laryngitis, tracheitis, operation tracheotomy.	−	...	...	+	+
17	503	31	*Haemorrhagic, purulent bronchopneumonia with abscesses.*	+	...	...	...	+
18	504	$1\frac{3}{12}$	Empyema, bronchopneumonia.	+	+	...	...	...
19	506	6	*Bronchopneumonia.*	+	...	...	...	...
20	508	36	Bronchopneumonia—Hodgkins lympho-granuloma.	−	+	...	+	...
21	523	6	Empyema, bronchopneumonia with abscesses.	−	...	+	+	...
22	525	$\frac{6}{12}$	Bronchopneumonia, catarrhal gastritis, enteritis and colitis—cultures overgrown with coliform bacteria.	−	...	...	...	...
23	531	$\frac{5}{12}$	Bronchopneumonia. Purulent meningococcal meningitis.	+	..	...	...	+
24	533	42	*Bronchopneumonia.* Syphilitic fibrosis with gummata of liver.	+	...	...	+	...
25	532	30	*Bronchopneumonia.*	+	...	...	+	...
26	534	39	*Non tuberculous and tuberculous bronchopneumonia.*	+	+	...	...	...
27	535	10	Pyaemia, pyaemic abscesses and bronchopneumonia, chronic purulent inflammation of sphenoidal air sinuses.	−	...	...	+	...
28	537	50	Bronchopneumonia.	−	+	...	...	...

Case.	Post-mortem No.	Age.	Macroscopic diagnoses.	*B. influenzae.*	*Pneumococci.*	*Streptococci.*	*Staphylococci.*	*Gram-negative cocci.*
29	538	52	Bronchopneumonia. Fibrino-purulent bronchitis.	+	...	...	...	...
30	540	36	*Bronchopneumonia, infected infarct of lung.* Pyaemia post-influenzal thrombosis of iliac vein.	+	...	+	+	...
31	544	20	*Pneumonia,* acute and chronic rheumatic endocarditis.	+	+	...	...	...
32	546	67	Bronchopneumonia, peritonitis, colostomy operation.	−	...	...	...	...
33	547	22	*Bronchopneumonia, empyema* — recent delivery.	+	...	+	...	+
34	556	42	Bronchopneumonia with abscesses, pneumococcal and syphilitic endocarditis, syphilitic aortitis.	−	+	...	...	...
35	560	48	Brown induration of lungs; syphilitic aortitis and acute and chronic rheumatic endocarditis.	−	...	+	...	...
36	561	$1\frac{8}{12}$	Bronchopneumonia, chronic streptococcal abscess of neck.	−	+	...	...	+
37	562	53	*Haemorrhagic pneumonia, Pachymeningitis interna haemorrhagica.*	+	...	+	...	...
38	564	4	Fibrinous laryngitis, tracheitis and bronchitis, operation tracheotomy.	−	...	+	...	...
39	565	30	*Organized and unorganizing bronchopneumonia.*	+	...	...	+	...
40	566	4	*Bronchopneumonia.*	−	...	...	+	+
41	575	36	*Bronchopneumonia,* acute and chronic rheumatic endocarditis.	+	...	...	...	+
42	576	51	*Empyema abscess of lung, bronchopneumonia.*	+	...	...	..	...
43	581	30	*Bronchopneumonia,* old bullet wound of right chest.	−	...	+	+	...
	1919							
44	3	33	*Membranous laryngitis, and bronchitis organizing bronchopneumonia. B. diphtheriae* present.	−	...	..	+	+
45	60	25	*Septicaemia, Lobar pneumonia, Mitral stenosis, acute and chronic rheumatic endocarditis.*	+	...	+	+	+
46	70	$\frac{18}{12}$	Bronchopneumonia.	−	...	...	+	+
47	72	32	*Septicaemia, pneumonia.*	+	..	+	...	...
48	73	7	*Early pneumococcal meningitis, bronchopneumonia.*	+	+	...	...	...
49	81	20	*Pneumonia, Mitral stenosis, acute and chronic rheumatic endocarditis.*	−	+	...	...	...
50	84	23	*Bronchopneumonia.*	+	...	...	...	...
51	85	2	*Slight muco-purulent bronchitis and bronchopneumonia,* tubercular meningitis with tuberculosis of lungs.	+	...	...	...	...
52	94	30	*Bronchopneumonia, organizing pneumonia, chronic bronchitis with abscesses of lung.*	+	...	...	...	...
53	88	24	*Pneumonia.*	+	+	+	..	...
54	90	57	*Bronchopneumonia.*	+	...	+	...	...
55	93	43	Tubercular meningitis.	−	...	+	+	...
56	91	65	*Bronchopneumonia* after operation for removal of bilateral ischial bursae.	−	...	+	...	...
57	92	20	*Bronchopneumonia,* cardio-vascular hypertrophy, chronic ascending nephritis —urogenital maldevelopment.	+	+	...	...	...
58	95	48	*Bronchopneumonia.*	+	+	.	..	+
59	96	50	*Bronchopneumonia, tracheitis.*	+	+	...	...	...
60	100	41	*Bronchopneumonia,* serous meningitis, Tetanus.	−	+	..	...	...

Case.	Post-mortem. No.	Age.		*B. influenzae.*	*Pneumococci.*	*Streptococci.*	*Staphylococci.*	*Gram-negative cocci.*
61	102	41	*Pneumonia*, mitral stenosis, chronic rheumatic endocarditis.	+	...	+	...	...
62	103	16	General fibrino-purulent peritonitis, chronic intussusception.	...	...	...	+	...
63	104	22	*Bronchopneumonia*, chronic rheumatic endocarditis and pericarditis.	+	+	...	...	...
64	105	65	*Haemorrhagic bronchopneumonia*, cardio-vascular hypertrophy.	−	...	...	+	...
65	106	40	Cerebral compression. Fibro-endothelioma of dura.	−	...	+	...	...
66	107	21	Pneumonia, brown induration of lungs. Mitral stenosis.	−	+	...	+	...
67	134	54	*Lobar pneumonia*, cerebral haemorrhage cardio-vascular hypertrophy.	+	...	...	...	+
68	146	36	*Bronchopneumonia.*	+	...	+	...	...

3. Serological Observations.

In view of the fact that the above bacteriological examinations show the constant presence of *B. influenzae* in the various lesions of the epidemic, the following serological investigations were undertaken with the idea of obtaining confirmatory evidence of the aetiological importance of this micro-organism.

It has been recognized for a considerable time that specific immune bodies against *B. influenzae* are to be found in the blood of convalescent influenza patients. These observations show that a considerable number of influenza patients have had their tissues invaded by *B. influenzae.* In the recent epidemic this has been repeated and confirmed by a number of workers, although a few have reported negative findings. Their observations are dealt with in a later part of the paper.

Complement Fixation Test.

In this test the blood serum of a number of convalescent influenza patients was tested against an antigen of *B. influenzae.*

Technique. The antigen was prepared by growing *B. influenzae* on a number of boiled blood agar plates over night. The luxuriant growth was carefully scraped off, weighed, and then emulsified in carbolic saline (9 per cent. saline plus 0·5 per cent. carbolic) in the proportion of 0·1 gm. to 100 c.cm. of saline. This antigen was then standardized against guinea-pig complement. Falling doses (0·25 c.cm. to 0·025 c.cm.) of the antigen were dropped by means of a Donald's pipette into the diluted guinea-pig complement (1 in 20), the volumes being adjusted so that the total volume in each tube was 1 c.cm. The tubes were then shaken to ensure complete mixing and incubated at 37° C. for one hour. At the end of this time 0·5 c.cm. of sensitized sheep's red corpuscles was added to each tube and the series replaced in the incubator. At the end of 20 minutes the largest quantity of antigen which just allowed complete haemolysis was noted and employed in the test proper.

In the actual test the total bulk of ingredients during incubation was 1·0 c.cm. as before, and falling quantities of the patient's serum (0·1 c.cm. to 0·0125 c.cm.) were employed. The serum was placed in the tubes, then the complement, and lastly the amount of antigen determined by the preliminary standardization. The tubes were then incubated at 37° C. for one hour and then 0·5 c.cm. of sensitized sheep corpuscles added (4–6 units of amboceptor). The tubes were then replaced in the incubator and the test read when the control sera gave definitely negative results. In the table below complete inhibition of haemolysis (i.e. positive) is indicated by 4, lesser degrees by a lower numeral.

Results. The following table shows that 12 out of 19 unselected influenza convalescents gave positive complement fixation tests:

		Strength of Reaction. Amount of Serum.			
No.	*Case.*	0·1 c.cm.	0·05 c.cm.	0·025 c.cm.	0·0125 c.cm.
1	Ha.	4	4	3	3
2	Wh.	0	0	0	0
3	H.	4	4	4	4
4	Tu.	4	4	3	2
5	Control	1	0	0	0
6	Control	0	0	0	0
7	Go.	0	0	0	0
8	Fr.	4	4	2	0
9	Hu.	0	0	0	0
10	Gr.	4	4	4	4
11	Sy.	4	4	0	0
12	McM.	0	0	0	0
13	Hu.	3	0	0	0
14	Wa.	4	4	2	0
15	Po.	0	0	0	0
16	Bl.	4	2	2	0
17	Wh.	2	0	0	0
18	Do.	4	4	4	4
19	Fi.	4	4	3	0
20	Ro.	4	0	0	0
21	Pe.	4	4	1	0

Agglutination Tests.

Agglutination tests were performed on the sera of the same patients. The test was carried out in small tubes or pipettes. The serum and the bacterial emulsion were then placed in a water-bath at 56° C. for one hour and the results read four to six hours later. Of 22 sera tested in this way 8 gave positive results in dilutions up to 1 in 100.

Therapeutic Effect.

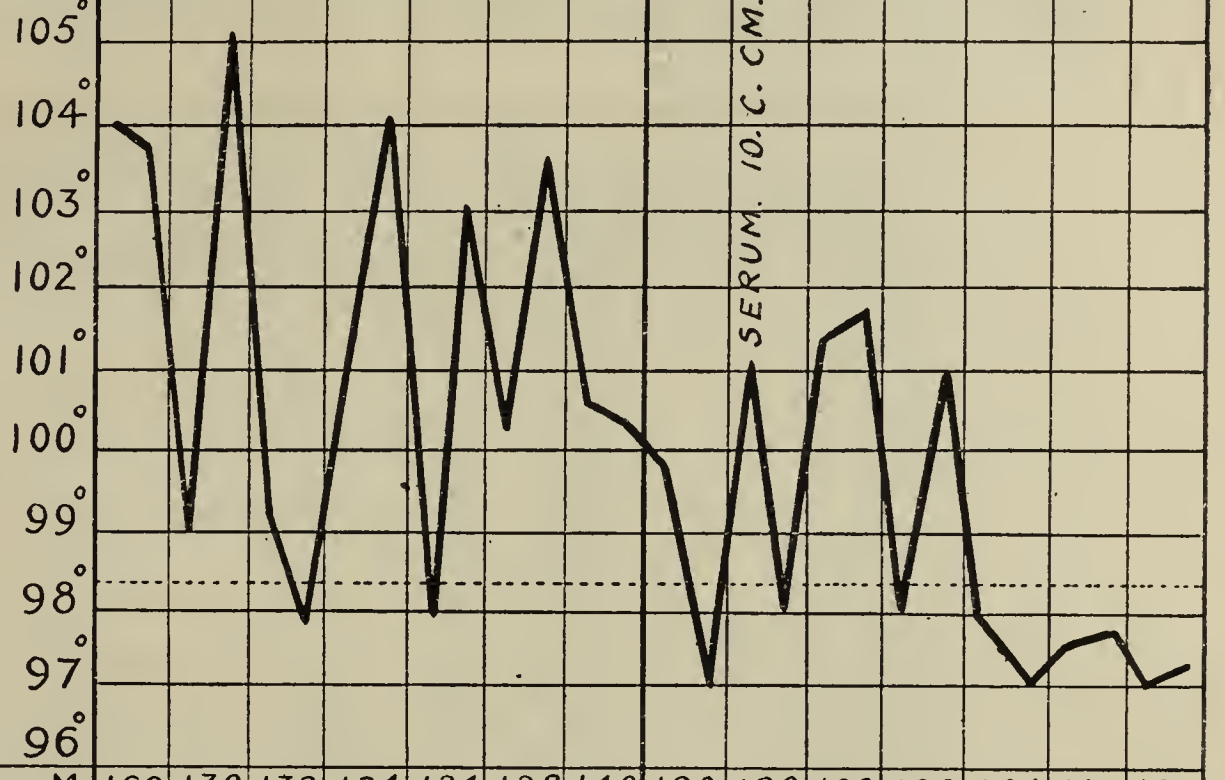
The curative effect of the pooled sera of influenza convalescents was tested on a number of acute cases of influenza. In most of the cases 20 to 30 c.cm. were used with considerable benefit to the patient. In some the effects were immediate, but in others the effects were slower. In all instances the patients said they felt better after the injection. It has been claimed that this is a non-specific effect and can be produced by the injection of any normal serum. Whatever the exact mechanism is, there can be little doubt as to the good effect as the accompanying unselected charts show.

MONTH	DECEMBER. 1918.												
DAY of MONTH	7	8	9	10	11	12	13	14	15	16	17	18	
F	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	
CASE Nº 1 — 103°			SERUM 20. C. CM.		SERUM 10. C. CM.								
102°													
101°													
100°													
99°													
98°													
97°													
96°													
PULSE M			106	120	108	100	101	98	94	98	82		
PULSE E			120	100	120	120	108	100	94	94	90		

MONTH	DECEMBER.								JANUARY.					
DAY of MONTH	24	25	26	27	28	29	30	31	1	2	3	4	5	6
F	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E
CASE Nº 2. — 105°									SERUM 10 C. CM.	SERUM 10 C. CM.				
104°														
103°														
102°														
101°														
100°														
99°														
98°														
97°														
96°														
PULSE M		80	96	88	88	100	100	72	76	88	86	84	92	96
PULSE E	86	90	100	104	96	100	96	80	96	88	88	88	80	
RESPIRATION M		20	28	28	24	26	28	30	34	32	28	28	26	24
RESPIRATION E	28	24	32	32	26	28	24	48	40	28	32	26	26	

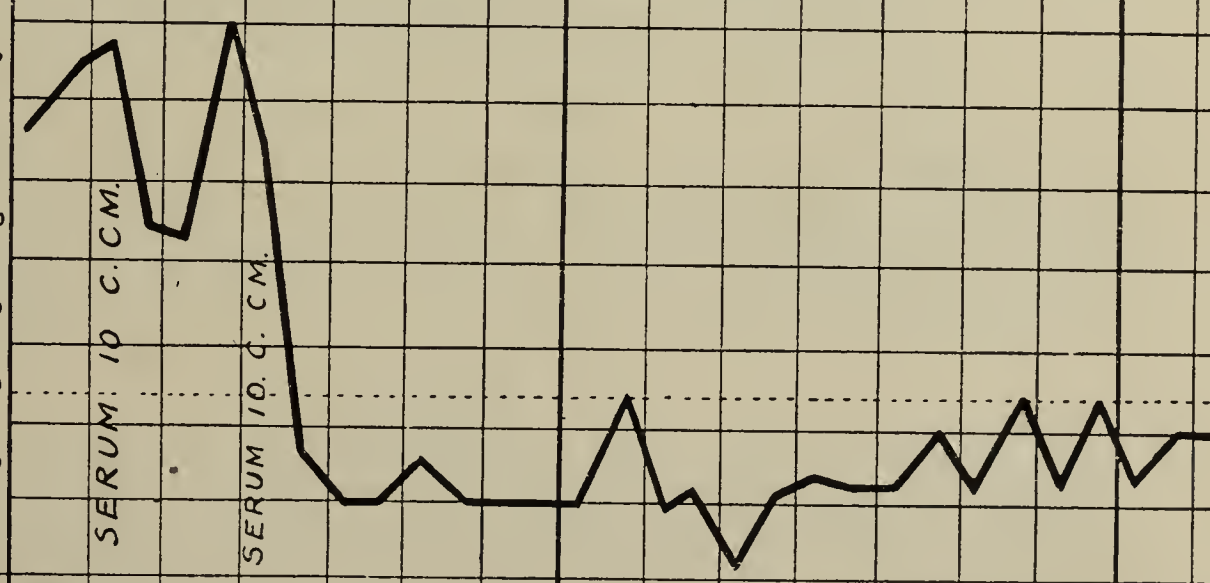
MONTH	DECEMBER 1918							JANUARY 1919						
DAY OF MONTH	25	26	27	28	29	30	31	1	2	3	4	5	6	7
DAY OF DISEASE	4	5	6	7	8	9	10	11	12	13	14	15	16	17
F	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E
PULSE M	160	130	132	124	124	128	140	120	120	100	120	104	100	104
PULSE E	160	144	104	160	148	140	104	112	96	146	120	108	108	
RESPIRATION M	36	50	48	40	44	40	48	44	40	48	36	40	40	28
RESPIRATION E	44	46	32	32	40	30	40	30	40	44	48	40	40	

CASE Nº 3



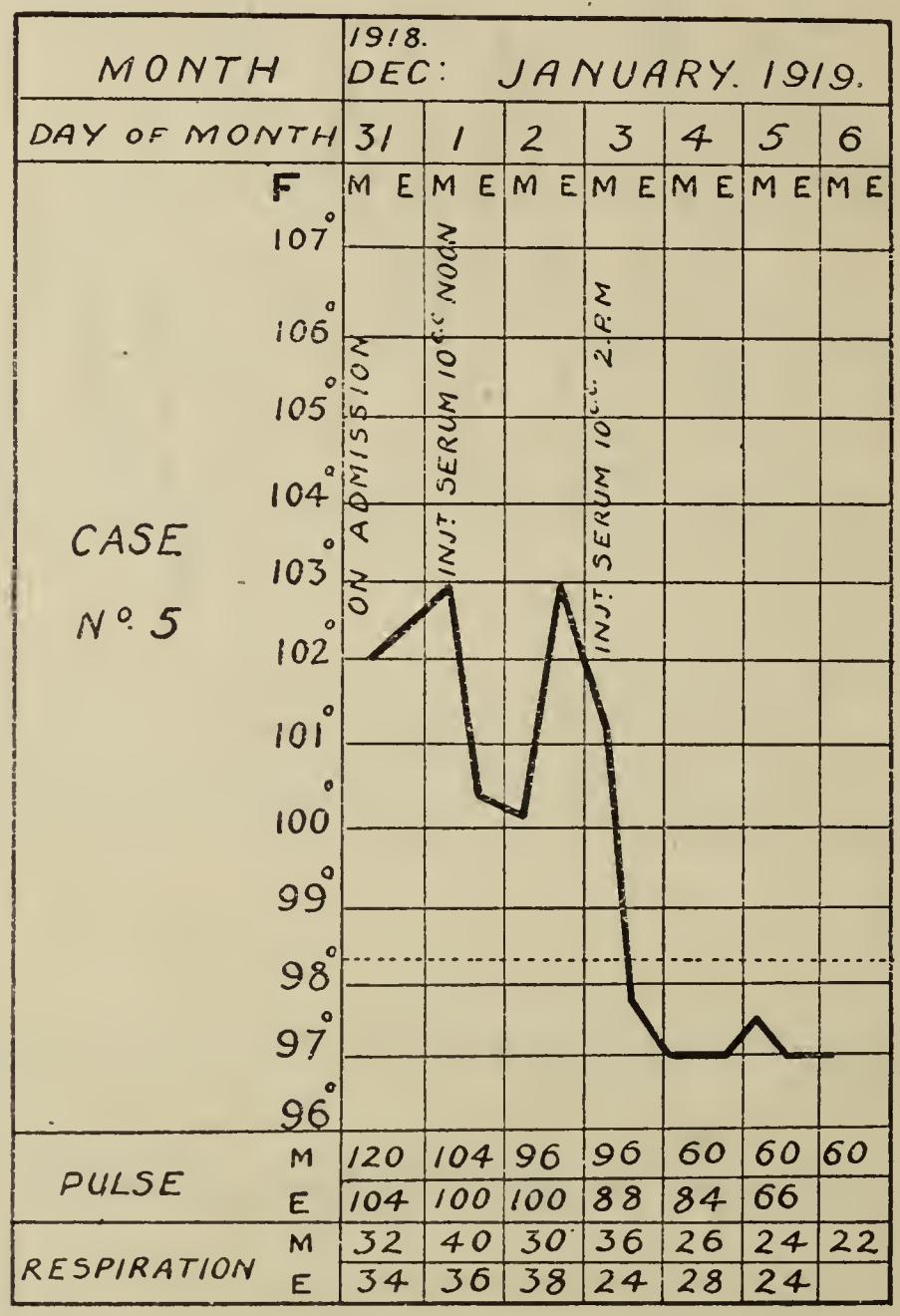
MONTH	DEC:	JANUARY.						JANUARY.								
DAY OF MONTH	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
F	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E	M E
PULSE M	120	104	96	96	60	60	60	60	60	82	80	98	84	96	84	102
PULSE E	104	100	100	88	84	66	68	62	62	80	76	80	88	88	96	
RESPIRATION M	32	40	30	36	26	24	22	26	20	20	18	26	20	20	22	24
RESPIRATION E	34	36	38	24	28	24	24	24	22	22	18	24	20	20	24	

CASE Nº 4.



4. Relation of the Micro-organisms to the Pathological Lesions.

It is, of course, necessary to correlate the bacteriological findings, in particular the incidence of *B. influenzae*, with the pathological lesions. For this purpose a short account of the gross and fine pathology of the tissues on which the bacteriological examinations were made is necessary.

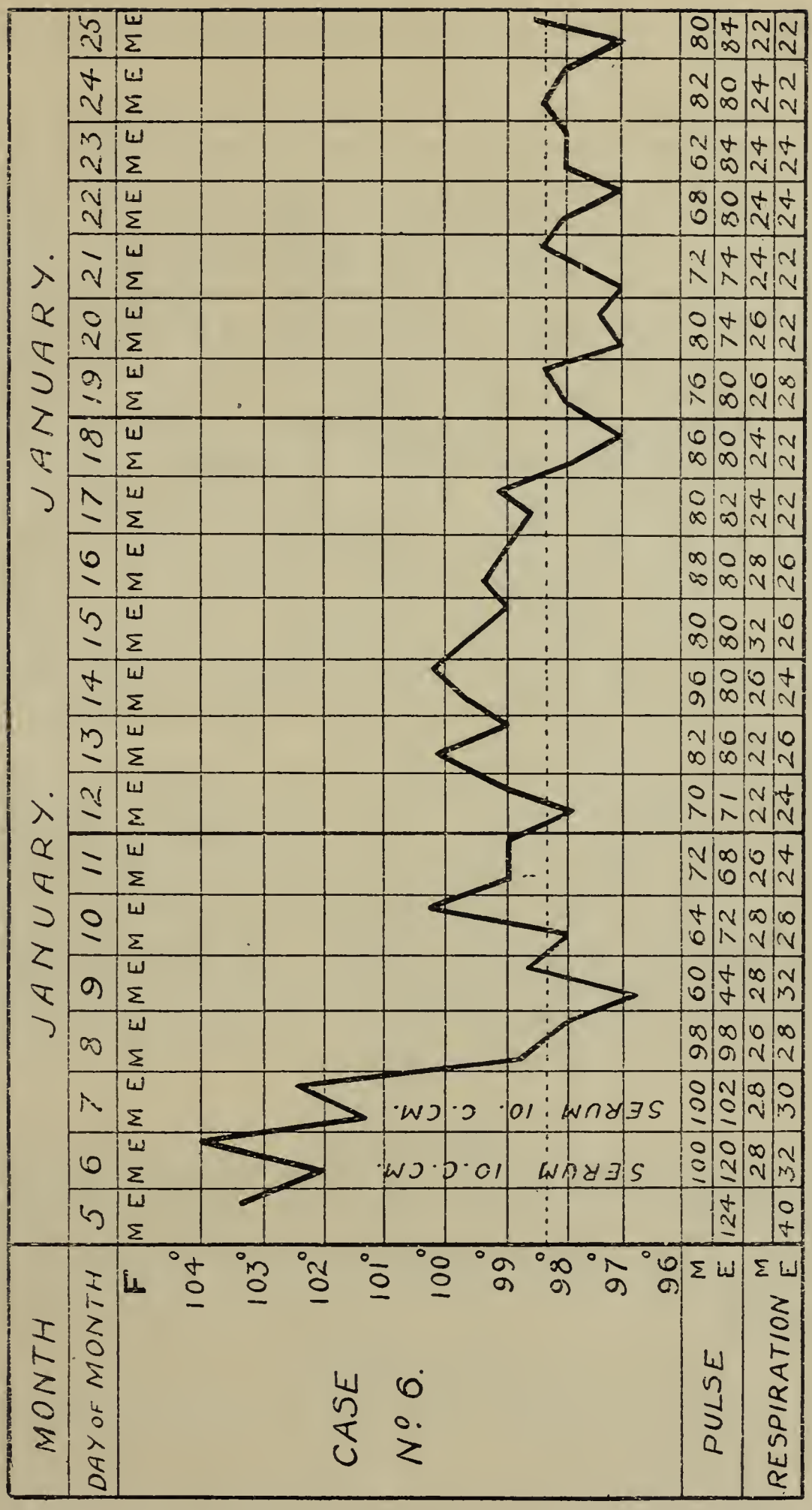


Morbid Anatomy.

Epidemic influenza is essentially an acute inflammation of the respiratory tract with involvement of the lungs. The most striking feature is undoubtedly the marked engorgement of the trachea and bronchi, and the general congestion and oedema of the lungs.

Trachea.

The inflammation and engorgement of the trachea is usually marked along its whole length and spreads down into the bronchi. In early cases the trachea may contain a considerable amount of frothy, more or less blood-stained, fluid. The mucous membrane is often covered with a layer of greyish muco-purulent material, and in places shows sub-mucous haemorrhages. In a few the deposit of muco-purulent material is thick and adherent so that the condition might well be described as membranous.



Lungs.

The lesions met with in the lungs vary according to the extent of tissue reaction and the length of the illness. The lesions may, therefore, be regarded as more or less progressive although all stages may be found in the same lung. On broad lines the types of lesions may be grouped together as follows :

(1) Oedema and congestion with haemorrhagic areas and broncho-pneumonia.

(2) Broncho-pneumonia with bronchitis and peribronchitis.
(3) Gangrene and lung abscess.

Dealing with these changes in the order named, the first is certainly the most characteristic appearance observed at autopsies. In early cases the lungs are found to be voluminous and of a dark-red colour. The pleura is as a rule smooth and often shows numerous submucous haemorrhages. The lungs feel soft and of a jelly-like consistence, while firmer nodules may be felt throughout the lung. In some this has been likened to a mass of frogs' spawn. On section, the lungs are usually dripping wet and exude a serous fluid more or less blood-stained; in others the exudate appears to be almost pure blood. Scattered throughout the lungs are firmer, usually haemorrhagic, areas, looking at times like infarcts. Alternating with these and the emphysematous portions of the lung are the oedematous areas. The fluid in the latter may be coagulated, giving rise to a firm gelatinous oedema. A small amount of grey pus can at times be squeezed from the bronchi, but usually they only contain a frothy fluid. In such lungs the effect is evidently a direct intoxication of the capillaries, which become permeable, first to the serum and later to the blood cells. The patients in such cases may almost be said to have been drowned in the exudate.

In cases of longer standing where the intoxication has been less severe, the broncho-pneumonia is more in evidence. The broncho-pneumonic areas vary in appearance, and show some tendency to coalesce so that in some lungs the lesion is difficult to tell from a lobar pneumonia. In more advanced cases still the bronchitis and peri-bronchitis are more in evidence. At times the lungs have a peculiar slimy appearance and pus can readily be squeezed from the bronchi. In some the greyish pneumonic patches had given place to necrosis and abscess formation.

Other organs.

The liver and kidneys frequently showed extensive parenchymatous degeneration. The spleen on the whole did not show much change, unless there had been a septicaemic condition before death. Degeneration of the heart-muscle was only observed on one or two occasions.

Histopathology.

With the idea of ascertaining how the pathological process became evolved, particular attention was paid to the earliest changes observed.

The trachea and bronchi of those individuals who died of acute intoxication showed engorgement of the capillaries with oedema of the submucous layer, while here and there, chiefly round the blood vessels, accumulations of small round cells were to be seen. The epithelium of the mucous membrane in some cases showed some slight proliferation, in others the cells were necrotic and in some places shed. Bacteria were usually to be found in large numbers in the mucous exudate; on the surface of the mucosa and among the epithelial cells. In many instances only small Gram-negative bacilli (*B. influenzae*) were to be seen. In others numbers of

different cocci were to be found, mostly in association with *B. influenzae.* In the actual tissues, bacteria were observed chiefly in the submucous exudate, evidently having penetrated through the damaged epithelium.

Bacteria were also demonstrated in the lymphatic channels of the bronchi, in these cases *B. influenzae* was frequently found, either alone or in association with Gram-positive cocci (streptococci and pneumococci). The affected lymphatics were distended with a serous effusion, which might or might not contain cellular elements In most cases the cells present were chiefly lymphocytes. (Figure 7.)

In the lungs the earliest changes observed were dilatation of the capillaries, and a pouring out of a clear albuminous fluid, which in places completely filled the alveoli. Even at this early stage haemorrhages had occurred, and in places the alveoli contained numerous red cells. Apart from red cells the cellular elements were few in number, one or two catarrhal elements with an occasional leucocyte. The epithelium of the bronchial mucous membrane might show some slight proliferation, with swelling of the mucous glands. The lumen might even contain plugs composed of shed epithelial cells, mucus and a few leucocytes. In the alveoli at this stage bacteria of any kind were rare; their presence in the bronchi, however, has already been commented on. As the lesion progresses the picture becomes more typically that of a broncho-pneumonia. The alveoli are plugged with a cellular exudate, and even fibrin, while the same is true of the bronchioles. Bacteria are more numerous at this stage, and in a number of the cases examined *B. influenzae* was found. In many, however, there is such a variety of bacteria present that it is impossible to enumerate the various types.

Summary.

Macroscopic and microscopic examinations tend to show that acute epidemic influenza is primarily an acute inflammation of the respiratory tract with involvement of the lungs, giving rise to a haemorrhagic oedema and broncho-pneumonia. At a very early stage of the infection bacteria are to be found in the respiratory tract but are rare in the lungs. Later the lungs contain large numbers of bacteria. The type of bacteria found corresponded closely with the cultural results, while the close relation between the lesions and the distribution of the bacteria suggests that they play an important part in the aetiology of the condition.

5. Attempts to Produce an Experimental Disease and the Question of the Presence of a Filter-passing Virus.

The following experiments were undertaken with the idea of ascertaining whether or not acute influenza could be transmitted to laboratory animals and to monkeys. Filtered and unfiltered exudates were made use of in the inoculations, in order to determine the possible presence of any filter-passing virus. The results obtained are given in tabulated form.

Table IV.

Attempted Transmission to Animals.

A. *Filtered Material.*

Material.	*Source of Material.*	*Date.*	*Animals.*	*Method of Inoculation.*	*Cultures.*	*Results.*
1. Nasal washings	Two early uncomplicated cases in special wards.	6/11/18	*Macacus rhesus* (2) Rabbits (2) Guinea-pigs (2)	Subconjunctivally 0·1 c.cm. Subcutaneously 5·0 c.cm.	Sterile	Nil, apart from a slight redness of the rabbit's conjunctiva.
2. Throat washings	Three acute early cases from the epidemic among the nurses.	20/11/18	*Mac. rhesus* (1) Hussar monkey (7)	As above, only 8·0 c.cm. given subcutaneously.	Sterile	Nil.
3. Lung juice	Three post-mortems showing characteristic lung changes.	15/11/18	*Mac. rhesus* Dog-faced monkey Rabbits (2)	As in no. 1.	Sterile	See note below.

B. *Unfiltered Material.*

1. Nasal and post-nasal secretion	Two acute uncomplicated influenza cases.	18/12/18	*Mac. rhesus* (2)	Direct swabbing of the nasal mucosae.	*B. influenzae* almost in pure culture.	Nil.
2. ,, ,,	One acute uncomplicated influenza case.	21/2/19	*Mac. rhesus* (2) Rabbits (2)	,, ,,	,, ,,	Nil.
3. Blood	Three acute uncomplicated influenza cases.	27/2/19	*Mac. rhesus* Rabbits (2)	... ...	Sterile	Nil.
4. Freshly isolated pathogenic culture of *B. influenzae*	... ...	25/3/19	*Mac. rhesus* (2)	Direct swabbing of nose with thick emulsion of the culture.	... ...	Nil.
5. ,, ,,	... ...	31/3/19	*Mac. rhesus* (1)	Left nares plugged with gauze soaked in thick emulsion.	... ...	Slight discharge from nose, but no rise of temperature.
6. Insufflation experiment	... ...	4/19	*Mac. rhesus* (1)	Tracheal insufflation of *B. influenzae* emulsion.	... ...	Slight discharge from nose, but no rise of temperature.

In this experiment only the dog-faced monkey showed any effects. This animal 10 days after the inoculation developed a running at the nose and a temp. of 132° F., but an uninoculated monkey in the next cage had a bad cold. From this the animal got gradually weaker and died 34 days from the date of inoculation.

A post-mortem examination showed accumulation of fluid in the pleural and pericardial cavities. The lungs showed no congestion, only a few consolidated areas. The spleen was slightly enlarged and showed a number of yellow areas. Bacteriological and histological examinations showed a recent septicaemia with miliary abscesses. The pericardial and pleural fluids were sterile. Blood cultures grew a coliform bacillus and staphylococcus. Passage inoculation (*Mac. rhesus* monkey) without result.

An analysis of Table IV shows that with one exception the inoculations produced no signs or symptoms which might be interpreted as an attack of influenza. The animals remained healthy even when observed over a long period of time independently of whether emulsions of sputum, lung juice, or cultures of *B. influenzae* were given.

The single exception mentioned above was a *Macacus cynomolgus* which ten days after inoculation developed a serous discharge from the nose and a rise of temperature. The animal gradually got weaker and died 34 days after it was inoculated. A post-mortem examination did not show any characteristic lung changes, and a bacteriological and histological examination of the tissues suggested that death was due to a secondary septicaemia. Passage experiments were negative.

The above experiments show that under ordinary conditions, it is impossible to transmit epidemic influenza to laboratory animals or monkeys. Negative results were obtained no matter whether filtered or unfiltered material was employed. Thus not the slightest evidence in favour of the presence of a filter passing virus of the disease was obtained.

Pathogenicity of B. influenzae.

In Table IV mention is made of inoculations of emulsions of *B. influenzae* on to the respiratory passages of monkeys. But previously to this, inoculations both of emulsions of the bacilli and of whole cultures were made into guinea-pigs and mice to test the pathogenicity of the various cultures isolated. A considerable degree of variation was found in the cultures. Some were practically avirulent, most had only a feeble pathogenicity, while a few were found to be highly virulent.

At first tubes of ordinary broth containing pieces of fresh rabbit kidney were used, and of 24 to 48 hours' old culture 2–3 c.cm. were injected into the peritoneal cavity of a guinea-pig. Out of a large number of strains tested in this manner three cultures were found which regularly caused the death of the animal in 24–48 hours. The symptoms produced were fairly constant. The animal shortly after the injection appeared to be ill, was disinclined to move and remained in a corner of its cage. As a rule the hair was ruffled and the animal refused to eat. Within 24 hours it was distinctly ill, unsteady on its feet, and still refused to eat. There was usually diarrhoea with the passage of blood-stained mucus. Death followed in 24 to 48 hours, or might be delayed for 72 hours. A post-mortem examination showed some subcutaneous oedema, while the visceral and parietal peritoneal surfaces were pink from the numerous congested vessels. The abdominal cavity contained a considerable amount of a slightly opalescent fluid containing flakes of fibrin, while at times there was fibrinous deposit on the surface of the liver. The intestines were distended with gas and not infrequently showed petechial haemorrhages. The other organs showed no apparent change. Microscopic preparations of the sero-fibrinous exudate showed the presence of small Gram-negative diplo-bacilli

with longer forms which were at times almost thread-like; only a few leucocytes were to be found. In mice a very similar train of symptoms were produced, independently of whether whole cultures or emulsified bacilli were employed.

The toxicity of surface growths of *B. influenzae* can be judged from the following experiment. A weighed quantity of the bacilli from the surface of a boiled-blood-agar tube was taken, emulsified in saline, and then injected intraperitoneally into mice of about 20 grammes weight.

Mice.	*B. influenzae strain.*	*Weight of bacilli.*	*Result 24 hours*
1	88	0·004 gm.	Dead.
2	88	0·002	Dead.
3	88	0·001	Dying.
4	R.	0·004	Dead.
5	R.	0·002	Very ill—dead next morning.
6	R.	0·001	Ill—recovered.
7	155	0·004	Dead.
8	155	0·002	Dead.
9	155	0·001	Very ill—recovered.

6. Question of the Production of a Soluble Toxin by *B. influenzae.*

Certain of the toxic symptoms, in particular the temperature collapse, diarrhoea, congestion of the gut were suggestive of the action of a toxin. In fact this resemblance to certain of the anaerobic toxins is quite marked. It was, therefore, thought advisable to test the effects of filtered broth cultures. At first the filtrates of several days old cultures were employed but without much success. Later, by use of 24-hour old cultures in boiled blood bouillon, toxic effects could be produced in rabbits and guinea-pigs on intravenous inoculation.

In the case of rabbits (1,500 gm.) the toxicity varied considerably, but as a rule 5 to 6 c.cm. of the filtrate was sufficient to make the animal seriously ill. Not infrequently samples of toxin were obtained of which the above amounts, or even less, killed the rabbits almost immediately. At times death occurred after an interval of three or four days, while animals which appeared to be seriously ill recovered completely. Where death did not immediately follow the injection, the animal became depressed and crouched in a corner of the cage and refused food. At times actual collapse was observed. Within a few hours frequency of micturition with the passage of brownish coloured urine and slight diarrhoea was not uncommon, while next morning, and particularly so in young rabbits, numerous subcutaneous capillary haemorrhages were seen on the ears. As a rule, the condition described persisted for two or three days, and was followed by complete recovery. An animal killed at this stage showed, as a rule, subcutaneous haemorrhages on the body and considerable lung changes.

In the case of the guinea-pig the average lethal dose was 2 to 3 c.cm., but with some batches of toxin the lethal dose was 0·5 c.cm., or less, death occurring almost immediately or after some hours. Perhaps the most suggestive train of symptoms produced

in a guinea-pig were those seen in an animal where death was caused in four or five hours. Immediately after the injection the animals became collapsed, their hair ruffled, and their temperature subnormal. Some time later a discharge of frothy mucus was observed at the nose and there was slight diarrhoea.

Lesions.

In the injected animals lesions, often considerable, were always to be found when the illness had lasted for some hours. In many instances what surprised one was that such extensive changes could have occurred in such a relatively short period. As the changes observed in rabbits and guinea-pigs showed great similarity one description will suffice for both. It might be as well to indicate, however, that on the average the lung changes in the guinea-pig were the more marked. But in both animals the lung changes were so constant that one might rightly assume that the influenza toxin or poison had a definite selective action on the respiratory mucous membrane.

Macroscopic Appearances.

On opening the animal usually a slight but definite oedema of the subcutaneous tissues was to be seen. More constant, however, were subcutaneous petechial haemorrhages. In young rabbits, as already mentioned, these were most numerous in the ears. The lungs usually showed that several small areas, or more rarely that the whole of one lobe was involved. In the affected parts one noted slightly sunken red areas alternating with paler lung tissue, i.e. areas of collapse and emphysema. In some definite haemorrhagic spots were observed. On cutting into the lungs frothy mucus welled out, this at times was distinctly haemorrhagic in character. The trachea frequently contained a considerable amount of this frothy mucus, and its wall showed some congestion. The pleural cavity did not, as a rule, contain more than a very slight excess of fluid, while the same was true with regard to the pericardium. On one or two occasions small haemorrhages were seen on the heart-muscle.

The abdominal cavity, however, usually contained a considerable quantity of a clear serous fluid. The abdominal organs were all congested, and not infrequently showed petechial haemorrhages. The intestines were distended and at times showed fairly large subperitoneal haemorrhages. The kidneys were either normal in appearance or soft and enlarged with subcortical haemorrhages. The suprarenals, particularly in guinea-pigs, were often greatly congested and of dark red colour, just as is seen after diphtheria toxin.

Microscopic Appearances.

Lungs: In sections the affected parts were seen to consist of alternating areas of collapse and emphysema. The collapsed areas gave quite a typical picture of broncho-pneumonia, proliferation and desquamation of the alveolar and bronchial epithelium, infiltration

of leucocytes and in places slight haemorrhages. In some areas the alveoli were filled with a coagulated albuminous fluid and at others with red blood corpuscles. Some of the alveoli in the emphysematous areas contained coagulated albumen and occasionally a few red corpuscles. In some of the veins thrombus formation had occurred.

Kidneys: The extent of the lesion in these organs varied considerably, but in a good number of the animals examined well-marked parenchymatous degeneration and necrosis of the epithelium was found together with small haemorrhages. On one or two occasions a slight increase of mononuclear cells was observed, particularly in the neighbourhood of the glomeruli.

Suprarenals: The degree of involvement was also considerable in these organs, in some the change was imperceptible, while in others the organ was the seat of a massive haemorrhage. In the majority the organ showed a well-marked congestion.

Liver: As a rule apart from congestion no definite change was observed.

It seems, therefore, that by the injection of the filtrates of broth cultures of *B. influenzae* one has been able to produce pathological changes in laboratory animals which present a close resemblance to those found in man after a fatal attack of influenza.

Nature of the so-called B. influenzae *toxin.*

Since the demonstration of a satisfactory method of producing toxic filtrates of *B. influenzae* the exact nature of the poison has been a subject for discussion. It is, of course, most important to decide whether the poison is a true toxin or not. If a true toxin such as that of *B. tetani* or *B. diphtheriae* it will conform to many of their characteristics, and its inoculation into suitable animals should lead to the production of anti-toxin. A considerable number of experiments were done on this point, but for the purpose of this article it will be sufficient to indicate a few of the chief ones.

The pure toxic effect of *B. influenzae* filtrates is clear and sharp, as the following observations show. In the preparation of the toxin the same technique was used in the majority of instances. In every instance 16 to 24 hours old cultures were used. The medium was ordinary nutrient broth to which 5 per cent. of sterile defibrinated sheep's blood was added. The medium was then heated in a water-bath to 100° C. for a few minutes. The coagulum was allowed to settle, and the clear medium pipetted off and distributed into fiat bottles; in this way as large a surface as possible is exposed to the air during the growth of the bacteria. Later a medium consisting of 5 per cent. of peptonized sheep's blood (Fildes) was used with equally satisfactory results. For inoculation a whole sloped agar (blood) culture of *B. influenzae* was used in the proportion of one tube to every 50 c.cm. of medium. The inoculated medium was placed in the incubator overnight. Next morning the medium was filtered through a small Berkefeld candle the efficiency of which had been previously proved.

Toxic effect on guinea-pigs.

Experiment I.

Guinea-pig.	*Filtered culture. Dose (intravenous).*	*Guinea-pigs about 400 grm. Result.*
No. 1	2 c.cm.	Died in a few minutes.
No. 2	2 c.cm.	,, ,,
No. 3	1 c.cm.	,, ,,
No. 4	½ c.cm.	Survived.
No. 5	2 c.cm. (culture no. 129)	Survived.

Experiment II.

Guinea-pig.	*Dose.*	*Result.*
No. 1	1·5 c.cm.	Died almost immediately.
No. 2	1·0 c.cm.	Very ill, found dead next morning.
No. 3	0·5 c.cm.	Ill, but survived.

On rabbits the toxic effect was very similar except that for rabbits of 1,500–2,000 gm. 5 to 7 c.cm. was the average dose required to produce an immediate fatal result. If death did not occur overnight the animal usually survived.

It has been urged that this immediate lethal effect suggests that we are not dealing with a true toxin as these have definite incubation periods. But recent researches on the toxins of the anaerobic bacteria of gas gangrene have shown that this is not always the case, and that toxins which produced true antitoxins can exercise an immediate lethal effect on intravenous injection.

Again, the effects on the suprarenals (congestion and haemorrhage) is very suggestive of a true toxic action, effects being very similar to that of *B. diphtheriae* toxin. The recent work of Cramer has shown that such effects on the suprarenal are produced by a number of specific bacterial toxins. The effects on the body temperature as indicated by the experiments given below also suggests a suprarenal effect. At first the temperature rises considerably and then falls again sharply if the animal is to die.

Experiment III.

		Temperature.			
Guinea-pig.	*Dose of Filtrate.*	*12 p.m.*	*2.30*	*4.30*	*16 hrs. later.*
No. 1	1·5 c.cm.	102·2	105·1	104·6	102·0
No. 2	1·5 c.cm.	102·0	105·0	104·2	101·8

Experiment IV.

This fever was almost entirely absent if a little antitoxin was given at the same time.

		Temperature.		
Guinea-pig.	*Dose of Toxin.*	*2½ hrs. later.*	*3½ hrs. later.*	*5 hrs. later.*
No. 1	2 c.cm. + $\frac{1}{10}$ c.cm. Immune sera	101·9	103·0	103·2
No. 2	,, ,,	101·2	102·2	102·4
No. 3 (Controls)	2 c.cm. without Immune sera	102·3	103·2	105·2

It has also been suggested that the toxic effects of these filtrates might be due to the presence of proteid degradation substances produced by the growth of the bacteria in the medium. But there

is no evidence so far that any substance of such a nature can produce these definite histological changes in the tissue. (Dale and Laidlaw (10), Abel and Kubota (11), and Fink (12).) Like true toxins the influenza poison is thermolabile, half an hour at 60° C. being sufficient to destroy the toxic action.

The crucial test, however, is whether an injection of the filtrate will lead to the formation of antitoxin or not. With this idea a number of rabbits were injected with increasing doses of the toxic filtrate over a period of from two to three months. Most rabbits thus treated were found to withstand at least one full lethal dose of the filtrate, while their sera *in vitro* had considerable power of neutralizing the filtrate. In fact, the sera of these rabbits had a weak but definite content of antitoxin against the filtrates of *B. influenzae* cultures as compared against normal rabbit sera.

Experiment V.

Rabbit.	*Dose.*	*Result.*
No. 1	7 c.cm. of toxin	No immediate effect found. Dead next morning with haemorrhages into gut and kidneys.
No. 2	7 c.cm. + 1 c.cm. of antitoxin	No symptoms at all.
No. 3	7 c.cm. + 1 c.cm. of normal rabbit serum	Very ill, recovered.

Experiment VI.

Rabbit.	*Dose.*	*Result.*
No. 1	6 c.cm. of toxin	Died in a few minutes.
No. 2	6 c.cm. + $\frac{1}{10}$ c.cm. of antitoxin	Soon collapsed, rapid breathing, and then slowly recovered.
No. 3	6 c.cm. + $\frac{1}{10}$ c.cm. of normal rabbit serum	No immediate effects, but some 30 minutes later breathing was slightly quickened and the animal rather restless, but this effect soon passed off.

From the above experiments it is evident that normal rabbit serum has a slight protective effect due to the presence of normal antitoxin. This is true of most toxin antitoxin experiments and to overcome this difficulty it is usual to use multiple doses of the toxin. But in the case of *B. influenzae* toxin this method of experimentation is not satisfactory, owing chiefly to the large doses necessary. This will be referred to later.

Experiment VII.

Guinea-pig.	*Dose.*	*Result.*
No. 1	2·5 c.cm. Filtrate + 0·5 c.cm. saline	Found dead next morning.
No. 2	2·5 c.cm. Filtrate + 0·5 c.cm. normal rabbit serum	,, ,,
No. 3	2·5 c.cm. Filtrate + 0·5 c.cm. Immune rabbit serum A	No ill effects.
No. 4	2·5 c.cm. Filtrate + 0·5 c.cm. Immune rabbit serum B	,,

As already indicated the neutralizing effects of these immune sera against multiple doses of the filtrate was not satisfactory. This failure of the antitoxin to act against multiple doses of the

poison is rather disappointing. It suggests that the antitoxin is weak and that possibly, in addition, some other poison to which no anti-substance has been elaborated, is present in the filtrate in small amounts. Thus when multiple doses of the filtrate are employed the second poison is so increased that a toxic dose is present. This seems quite a likely explanation, as the hyper-immunized animals at times succumbed to three or four lethal doses of the filtrate. In addition, this immune serum when given along with the living culture can prevent an infection in the guinea-pig. Both culture and serum are given intraperitoneally.

The toxic filtrates, therefore, of cultures of *B. influenzae* present many points of similarity with true toxins. The only real objection is the failure of the antitoxin to act in multiple doses. It is hoped, however, that further experiments will elucidate this point. The therapeutic value of a good antitoxic serum in cases of acute influenza cannot be overestimated.

7. Relation of the Filter-passing Viruses to Influenza.

In the following section an attempt is made to summarize recent researches on the subject of filter-passing organisms so far as they concern the aetiology of influenza. The literature has been dealt with critically in order to ascertain to what extent these various claims have been substantiated. At the same time the opportunity is taken of placing my own experiences in line with those already published.

Filter-passing microbes as the cause of acute influenza have been described by a number of workers. The unanimity with which the claims have been brought forward alone carries considerable weight, but the trivial nature of most of the papers and the lack of experience in some of the investigators compels a certain degree of caution in accepting much of the work. This is particularly true when one recognizes the fact that the alleged discovery of an ultramicroscopic virus absolves the discoverer from the necessity of producing a description of the morphology of the microbe.

Kruse (13) appears to have been one of the first to advance the concept that influenza might be due to an ultramicroscopic virus. In 1914 as the result of one experiment he claimed that he had proved that common colds were due to a filtrable virus. The diluted and filtered nasal secretion from a case of acute nasal catarrh was placed in the noses of 30 members of his class. Fifteen of these developed colds and only one of the uninoculated controls. But it is obvious that if his assistant had a cold that this disease must have been epidemic at the time. This completely vitiates the experiments. In addition, the author made no attempt to show that he was dealing with a living virus. In fact, the same result could well have been produced by the irritant effect of an injection of weak formalin in the noses of persons exposed to infection. That colds are due to the *Aphanozoa coryzae,* as he termed his virus, has not been proved. Foster (14, 15) in America repeated and supplemented Kruse's work and claimed, in addition, that he had succesfully grown the virus. Cultures were obtained from the

filtered nasal secretions of ordinary colds. He employed Noguchi's medium and observed a cloudiness in the medium just above the haemolytic area in 24 hours' time. Smear preparations of the cloudy fluid after prolonged staining by Giemsa showed, under the microscope, minute coccoid bodies which were accepted as microbes. Further experiments by Dold (16) working in a German educational institute in Shanghai gave similar results in the case of Chinese students who lived together. But another experiment with hospital patients gave negative results. Dold also grew the virus in Noguchi medium; in the stained preparations nothing was found, but under the dark ground illumination numerous minute particles with active movement were seen. This culture, when placed in the nose of three individuals, produced in two increased nasal secretion and a slight cough. The author thus claimed that he had discovered the virus of common colds as an injection of the culture produced the characteristic lesion in man.

But any one who has worked to any extent with Noguchi medium will at once recognize that the growing cloudiness described is of little or no importance and is often to be seen in uninoculated tubes, while the multitude of minute particles found in the medium after prolonged staining or under the dark ground is only to be expected in albuminous fluids.

One is of opinion, therefore, that the description of these natural particles is sufficient to justify a complete rejection of the claims.

The above researches have undoubtedly influenced the trend of thought with regard to influenza, and for this reason have been given in considerable detail. Kruse (17) again in 1918 put forward the idea that epidemic influenza was due to an ultra-microscopic virus. v. Angerer (18) found similar bodies to those described above in cultures which had been made from the filtered blood of rats which had been inoculated with influenza sputum. No further experiments appear to have been done with this 'Virus'. Leschke (19) found particles with active Brownian movements in filtered lung juice of fatal influenza cases. He also attempted to transmit the disease to others by spraying the throat with the filtered lung juice. The first attempt was negative, but a later attempt in September 1918 was successful. The number of people operated on is not stated. No attempt was made to exclude the possibility of extraneous infection, and no attempt was made to show that he was dealing with a living virus. The same arguments can be used against Selter (20) who filtered the pooled throat washings of five cases of influenza and then along with his assistant inhaled the sprayed filtrate. Next day he had a cold and headache but no fever. His female assistant had some slight disturbance and slight fever, 37·6° C. But by no stretch of imagination could one accept the symptoms described as those of acute influenza.

Particles showing Brownian movement were described by Fejes (21) in ascitic broth which had been inoculated with filtered influenza sputum. A series of monkeys were inoculated with his cultures, injections of heated culture being used as controls. Four monkeys which had received the unheated culture developed an illness, and died in from nine to twenty-four days. The controls

remained healthy. Post-mortems revealed in these animals a condition of haemorrhagic sepsis, haemorrhages being found in all the tissues of the body. No mention is made of histological or bacteriological examinations, one is therefore at liberty to assume that his filters were not so bacterium-proof as he believed, and no attempt is made to exclude ordinary bacteria as the cause of death in his positive experiments. Prell (22) described granules in sections of influenza lungs which he called '*Aenigmoplasma influenzae*', and claimed that they were the cause of influenza.

In France Nicolle and Lebailly (23) came to the conclusion that influenza is due to a filtrable virus which is to be found in the respiratory tract but not in the blood. Their experiments are not very convincing. Seven observations were made on man and on monkeys with three positive results. Filtered influenza sputum was inoculated subconjunctivally into a monkey which later showed depression, wasting, and diarrhoea. The same filtered sputum was inoculated into a man subcutaneously, who six days later developed what appeared to be the classical signs of influenza. In another man a similar injection produced a mild influenza. The other experiments were negative. As with the German workers the above authors made no attempt to show that their virus was anything other than the irritant effect of the sputum injection, an effect which might have been produced by sputum from a non-influenzal case.

In Britain, Bradford, Bashford, and Wilson (24) claimed to have cultivated the filtrable viruses of a number of infections including influenza. Their work, however, owing to the criticisms of Arkwright (25) appears to have been withdrawn.

Gibson, Bowman, and Connor (26) working in the Army laboratories in France carried out a large number of experiments, and on those claimed somewhat similar findings in the case of influenza. Owing to the unfortunate death of one of the workers the work was not completed. They also noted the spreading cloudiness in the Noguchi tubes which they inoculated, and carried on the 'Virus' in series. They state that two *Macacus rhesus* monkeys inoculated subconjunctivally and intravenously with filtered influenza sputum developed on the second and seventh day an acute illness which was considered to be experimental influenza. Autopsies showed a haemorrhagic exudation in the lungs and otherwise comparable to lesions of influenza. Coccoid bodies were recovered in culture from the kidneys of the animals, and on inoculation reproduced the disease. They failed to grow these bodies from the kidneys of normal animals.

The coccoid bodies described by the above writers are without much doubt similar if not identical with those described by other workers as the filtrable virus of influenza. Their descriptions of the experimental lesions are not always clear, and with regard to passage experiments the only definite statement is that the animal's lungs showed some haemorrhagic areas. I think, however, that these writers paid too much attention to the lesions found in monkeys, animals which we know are extremely liable to inflammatory lung conditions of all kinds.

Completely negative filter-passing experiments have, on the other hand, been obtained by a number of workers, and in all probability many more have refrained from publishing purely negative results. Thus Lister and Taylor (28) working on volunteers obtained uniformly negative results. These volunteers were removed to an island 500 miles away where there was known to be no influenza, and where the possibility of spontaneous infection could be eliminated. Nasal washings from acute influenza cases, both filtered and unfiltered, were introduced into the nasopharynx of these men and also into monkeys. Those men receiving the unfiltered material developed a typical influenza, while the filtered material produced no effect on man or monkey. Using extracts of lungs from fatal cases of influenza under very similar conditions, Wahl, White, and Lyall (29) obtained only negative results, while a series of similar experiments undertaken for the American Navy were also negative.

My own experience as indicated in the first part of the paper agree with the negative findings. In only one experiment out of many was the injection of filtered material followed by any abnormal symptom. In this instance a *Macacus cynomolgus* monkey, which received an inoculation of 5 c.cm. of the filtered lung juice obtained from three fatal influenza cases, became ill ten days later. It developed a running at the nose and a temperature of 103·2 F., but an uninoculated monkey in the next cage had a bad cold. The animal gradually got weaker and died 34 days after the inoculation. The post-mortem showed an accumulation of serous fluid in the pleural and pericardial sacs. The lungs were not congested and showed only a few collapsed areas. The spleen was slightly enlarged and showed a number of small yellow areas. Bacteriological and histological examinations revealed a recent septicaemia with miliary abscesses. Passage experiments were negative. This apparently was a spontaneous cold in a monkey complicated by a septicaemia.

In conclusion, it must be held that the claims to have discovered a filtrable virus of influenza are unconvincing, and that the minute bodies observed in cultures have not been differentiated with certainty from those inanimate particles present in all albuminous fluids. At the same time the human experiments are unsatisfactory in that the subjects were not isolated and the disease atypical. Similarly the infections produced in animals were atypical and the lesions indefinite. Without additional proof it is therefore impossible to accept the production of an experimental influenza by a filter-passing virus.

8. Relation of *B. influenzae* to the late Epidemic.

In the preceding chapter an attempt was made to show that the various claims so far advanced with regard to the discovery of a filter-passing virus of influenza have not been substantiated. While in view of the fact that the present research has indicated that *B. influenzae* is present in the majority of cases of influenza,

and bears a close relation to the lesions both spontaneous and experimental, it is thought advisable to correlate these findings with the results obtained by other workers. For this purpose a review of the literature is given below. At the same time one will be able to judge how far *B. influenzae* as the causative agent of epidemic influenza conforms to Koch's postulates.

Previous to the late outbreak of influenza, if one had asked what was the aetiological agent of acute influenza the answer in nine cases out of ten would have been *B. influenzae* Pfeiffer. The minority objected to Pfeiffer's claims mainly on account of the fact that this bacillus could not be demonstrated in certain epidemics which appeared to be true influenza, and that it was frequently present in other diseases. The explanation of this we shall return to later. The failure on the part of many workers satisfactorily to demonstrate this bacillus in the early part of the epidemic increased the number of dissenters. In Germany, likewise, the nature of the epidemic caused a good deal of discussion both on account of the aberrant findings of the bacillus and of the clinical features. A census of the opinion of the chief bacteriologists and others was obtained by means of telegrams without achieving any very definite result. These inconsistent findings are fully elaborated in the review on the subject of influenza in the *Medical Supplement* of 1918 (30).

Thus it was not to be wondered at that many considered that the epidemic was something other than acute influenza. But a more careful consideration of the clinical and bacteriological features of the outbreak modified this opinion considerably as time went on. Many individual workers, including the writer, went through the same period of uncertainty as to the part played by *B. influenzae.* In fact, the epidemic can be divided into two stages, a first, in which *B. influenzae* was seldom demonstrated, and a second. in which this bacillus was demonstrated with great regularity. This fact is not attributable to any alteration in the epidemic itself, but to the application of new methods for the demonstration of the bacillus of influenza.

B. influenzae Pfeiffer is by no means easy to demonstrate in the lesions and sputum of influenza cases. It is a small bacillus with no distinctive staining reactions such as those of the tubercle or diphtheria bacillus. Thus, unless present in very large numbers it is almost certain to be missed if microscopic means are alone employed. This diagnostic difficulty is further complicated by the inability of the bacillus to grow on ordinary laboratory media.

Until quite recently it was always believed that the addition of a little blood to any of the ordinary laboratory media was sufficient to obtain a growth of *B. influenzae.* This, however, is only true in a restricted sense, as the growth obtained is minimal and the largest colonies mere points on the surface of the medium. This type of medium was used early in the epidemic with most unsatisfactory results. The discovery, or rather rediscovery, that unaltered blood did not provide the best medium completely changed the aspect of affairs. Immediately following on the demonstration that the addition of a little blood altered by heating,

or by digestion, to ordinary media enabled luxuriant growths to be obtained, the problem of the demonstration of *B. influenzae* was solved. From that time onwards careful workers reported the presence of *B. influenzae* in the majority of cases of influenza.

For reasons indicated on the previous pages the bacteriological observations made in the beginning of the epidemic on the incidence of *B. influenzae* are not of much value. We have, therefore, confined most of our remarks to those observations made at a later date. By this time the majority of bacteriologists were familiar with the use of the new selective media.

Incidence of B. influenzae.

Uncomplicated influenza. In the spring of 1918 Matthews (6) by means of his special medium found *B. influenzae* in all his cases of acute influenza (12), Eyre and Lowe (73) in 12 out of 14 sputa, Fildes, Baker and Thompson (32) in 12 out of 15 uncomplicated cases, and McIntosh (33) in an acute epidemic among nurses in 8 out of 12, and Martin in 76 per cent. (34). More recently Schorer (35) in 81 cases found the bacillus in 91·5 per cent. On the other hand, Little, Garofalo and Williams (36) failed to find *B. influenzae* in the exudates from the upper air passages, and used this as an argument against the influenzal nature of the epidemic. The negative results, however, are to be explained on the grounds that they used unsuitable media (Legumine serum agar). While the numerous negative findings among German workers—Gruber, Kolle, Benda, Schmore, Friedemann, Kruse, &c.—as reported by Friedberger and Konitzer (37) are probably due to the same cause.

Broncho-pneumonic cases (*ante mortem*). Although, clinically, most cases of acute influenza can be roughly grouped into two divisions, those with and those without pulmonary symptoms, a very careful examination of the chest will in the great majority of cases show some form of lung involvement, so that the above classification is quite arbitrary and really one of degree.

Among the first workers to demonstrate the frequence of *B. influenzae* were Hammond, Rolland, and Shore (4). They state that in an epidemic of purulent bronchitis in France during the winter of 1916–17 *B. influenzae* was present in a large number of the cases. In a series of 20, the bacillus was found in 18 of these by microscopic and cultural examinations. They therefore considered that *B. influenzae* was the cause of the epidemic and give the following reasons: The almost constant presence of the organism in the sputum and pus of affected bronchioles, and the fact that in some cases it occurred quite apart from the presence of any other organism. Shortly after this Abrahams, Hallows, Eyre and French (5), grew *B. influenzae* from 7 out of 8 cases of purulent bronchitis.

In the true epidemic period Eyre and Lowe isolated this bacillus in 12 out of 14 sputa (73), Fildes, Baker, and Thompson (32) in 45 out of 106 cases, McIntosh (33) in 42 out of 69, while Messerschmidt, Hundeshagen, and Scheer (38) demonstrated Pfeiffer's bacillus in 48·9 per cent. of the June–July epidemic and in 90 per

cent. of the September epidemic. Pfeiffer (39) himself states that in 217 sputa sent with the clinical diagnosis of acute influenza, the bacillus was present in 51·6 per cent. Dick and Murray (40) found it in 63·2 per cent. of their cases, Park (41) in 80 per cent., Medalia (42) in a large series of 2,279 cases in 76·8 per cent., and Schorer (35) in 93·8 per cent. of 242 cases. Thus, *B. influenzae* Pfeiffer may be taken as being present in from 50 to 90 per cent. of all cases of influenza showing pulmonary complications.

Blood. In the blood *B. influenzae* is only present on rare occasions, and then usually in association with other bacteria. Fleming (43) and Medalia found it a few times, but most workers have got uniformly negative results (Fildes, Baker and Thompson, McIntosh, and Dick and Murray). In the heart-blood of post-mortem cases Wilson and Steer (44) found *B. influenzae* 12 times. Similar findings have been recorded from time to time in empyemata, pericarditis, meningitis, &c.

Post-mortem material. In the beginning of the influenza epidemic almost the only organisms of importance which were demonstrated in the lung lesions were streptococci and pneumococci. Much time and trouble was spent without obtaining any evidence as to the presence of *B. influenzae.* The introduction of selective media, as already indicated, resulted in the demonstration of the bacillus in a large number of the cases as can be seen from the figures given below. Thus Fildes, Baker, and Thompson, Patterson, Little, and Williams (45) record its presence in almost all post-mortem material, Muir and Wilson (46) in 30 fatal cases leading them to remark that *B. influenzae* at least plays a very important part in the production of the lesions. Tytler, Janes, and Dobbin (48) in 60 out of 67, and Keegan (49) in 82 per cent. Olsen found the bacillus in 75 per cent. of the lungs of 220 post-mortems. Lister (47) examined the lungs of 56 natives who had died during the epidemic in the gold mines at Witwatersrand (South Africa) and found *B. influenzae* in 53. The writer found the bacillus in 36 out of 48 cases, and in a second series in 94 per cent. The negative findings have been recorded particularly by a number of German pathologists whose names have already been noted.

Histological examinations have shown that *B. influenzae* is found in close relationship to the pathological lesions. The bacillus is to be found in the whole of the respiratory tract right down into the smallest bronchi and alveoli of the lungs. As a rule, there is no general invasion of the tissues such as is the case with streptococci and pneumococci in fatal cases. The bacilli are usually to be found, however, in the lymphatics of the sub-mucosa, and from time to time in the heart-blood and empyema pus. The serous exudates in the neighbourhood of inflamed tracheal and bronchial glands may also contain the bacillus. (Dietrich (50), Wolbach (51) and personal observations.) *B. influenzae*, therefore, in its tendency to remain on the surface without invading the tissues, may be likened to *B. diphtheriae.*

In other diseases. B. influenzae has been demonstrated in the respiratory tract of a variety of other conditions such as measles, whooping cough, phthisis, &c., but at present the exact frequency

is difficult to estimate as the figures given by different workers vary greatly. In such conditions as measles where the highest findings have been obtained (Park (41) 90 per cent.) the differentiation from *B. pertussis*, Bordet, must complicate matters. In trachoma haemophilic bacilli indistinguishable from *B. influenzae* have been found in as high percentage as 50. Here, however, we cannot say that *B. influenzae* does not play an important rôle, as personal observations have shown that it is a not uncommon cause of acute purulent conjunctivitis. But any estimates based on patients in hospital wards, and especially during an epidemic, tend to be fallacious, owing to the rapidity with which one carrier of the bacillus can infect the remainder of the ward. The same, of course, is true with any of the microbes to be found in the respiratory tract. In the case of haemolytic streptococci Cole and MacCallum (52) found that in the measles ward the incidence was 56·5 per cent. although on admission the incidence was 11·4 per cent.

In the normal individual. The true incidence of the extent to which *B. influenzae* is normally present in the nasopharynx of healthy individuals is also difficult to estimate, as most of the figures available are based on observations made in an epidemic period. It will, therefore, be necessary to wait for some time until accurate and reliable figures can be obtained. Fildes, Baker, and Thompson examined 71 apparently healthy men and obtained 15 positive cultures—21·1 per cent. Later, Fildes examined 76 boys on entering a training establishment and found 18 positive. In February to March, 1919, he made cultures from the throats of 177 healthy men in a barracks and obtained 64 per cent. positive. In a training-school for girls, Wadsworth (53) reports that *B. influenzae* was cultured from the throats of 3 of the inmates, who numbered 461. Pritchett and Stillman (54) found that 43 per cent. of normal individuals in the personnel of a large hospital carried the bacillus. But one is hardly justified in calling any of these individuals a normal sample of the population.

Association with other bacteria. We have already drawn attention to the well-known fact that various pathogenic bacteria, in particular cocci, are present in most catarrhal conditions of the respiratory passages and to a lesser extent in the healthy state. Therefore, any statement of their mere presence or absence can not be of much value. The difficulty, however, has been to devise a simple procedure which will give some sort of idea of the actual predominence of any particular organism. For some organisms film diagnosis is very satisfactory, for others, such as *B. influenzae*, it is almost useless. On the whole plate cultivation with a rough estimation of the number of colonies gives the best all round results.

The organisms most commonly associated with *B. influenzae* are streptococci, pneumococci, staphylococci and various Gram-negative cocci (*Micrococcus catarrhalis*, *M. pharyngis siccus*, and meningococci).

It has been shown, however, that even in the same locality the predominant associating microbe has changed from time to time. At one period it may be pneumococcus, at another haemolytic streptococci and at another Gram-negative cocci. In this country

streptococci were more frequent than pneumococci, although in America the opposite seemed to be more frequent.

Thus Fildes, Baker and Thompson found haemolytic streptococci in the majority of their post-mortems, Opie, Freeman, Blake, Small, and Rivers found that its incidence varied from 4 to 14 per cent. according to the type of case. Cole and MacCallum also found haemolytic streptococci in a large number of cases in an epidemic of pneumonia, and frequently in association with *B. influenzae*. Lister (47) found pneumococci present in 42 out of 50 lungs examined.

Pneumococci were found to be present by Opie (55) and his co-workers in from 53·6 to 69·8 per cent., and by Small and Stangl (56) in 72·7 to 84·4 per cent. The writer found pneumococci in about 30 per cent. and streptococci in 30 to 37 per cent., about one-half of which were of the haemolytic variety.

Staphylococci are not so infrequent as one would imagine, in some instances they appeared to spread in epidemic form, and to have been so frequent that it was suggested (Patrick (57)) that they were of some aetiological importance in epidemics of broncho-pneumonia. The high frequency of Gram-negative cocci in certain instances has led to the expression of a similar opinion. In addition, these cocci have been shown by microscopical examination to be in close relation to the histological changes found in the bronchi and in the lungs. Fletcher (58) demonstrated the presence of meningococci in large numbers in association with *B. influenzae* in the lungs of 11 fatal influenza broncho-pneumonias.

From time to time other organisms such as the pneumo-bacillus, pleomorphic streptococci, *B. proteus*, &c., have been found in considerable numbers.

Pathogenicity of B. influenzae.

For man. Numerous attempts to demonstrate a direct pathogenic effect of *B. influenzae* for man, by the production of acute influenza by means of pure cultures of the bacillus, have so far been almost uniformly unsuccessful. At most an evanescent or fleeting pyrexia with slight catarrhal symptoms have been produced. Lister and Taylor (28) introduced living suspensions of this bacillus and of two varieties of cocci into the noses and mouths of 9 men, and only one of those who received *B. influenzae* fell ill. The authors would not, however, ascribe this illness to influenza. Rosenau (59) treated 19 men with 13 strains with negative results. Wahl, White, and Lyall (29) and McCoy were equally unsuccessful. Park (60) in a recent communication reports two such instances and a third, in which, although no symptoms were produced, the subject became a carrier of *B. influenzae*. The most reliable means, therefore, left at our disposal are indirect procedures, namely, the demonstration of immune substances in the blood of affected individuals.

The presence of specific antibodies in the sera of patients is proof that at some not very remote period the tissues have been invaded

by that particular organism. But the converse is not exactly true, as in the case of those bacteria which invade the tissues only slightly and injure by the production of toxins the antibody response to the bacterial proteid is small. However, in spite of this, *B. influenzae* invades the tissues sufficiently to give rise to specific antibodies. Positive agglutination reactions have been obtained by a number of workers, while a few have failed. Fleming (61) obtained positive results in all of his 21 cases; Spooner, Scott, and Heath (62) got comparable results; but Materna and Penecke (63) only got positive results in 35 per cent. of their cases.

Complement fixation tests have also given numerous positive results, particularly in the hands of Howell and Anderson (64), Kolmer (65), Rapoport (66), and Wollestein (67). My own researches confirm these findings.

For animals. The pathogenic action of *B. influenzae* upon animals has been studied by many. In general it may be said that animals can resist large doses. Even massive doses introduced into the peritoneal cavity only cause a slight inflammation. The bacteria disappear rapidly and the animal recovers.

On the other hand, liquid cultures often exhibit marked pathogenic action. The condition produced is that of septicaemia together with a profound intoxication. There is usually diarrhoea and oedema of the subcutaneous tissues. Unfortunately subcutaneous or intraperitoneal injections do not lead to any regular lung changes. Attempts made to produce these by intra-tracheal injections of liquid cultures and suspensions of the bacilli have in most instances ended in failure. Quite recently, however, Blake and Cecil (68) by means of a culture whose virulence had been raised by passage through a series of mice were able to produce in monkeys an infection of the upper respiratory tract which could be complicated by acute sinusitis, tracheo-bronchitis and broncho-pneumonia. They used first or second subcultures on blood agar or in boiled blood of the *B. influenzae* recovered from the peritoneal exudate of monkeys with *B. influenzae* peritonitis.

In their first series 12 monkeys were inoculated by swabbing the naso-pharynx with a swab dipped in the culture, or by directly dropping the liquid culture into the nose by means of a pipette (1 c.cm.). In the animals successfully inoculated there was a rise of temperature with symptoms of coryza, blinking of the eyes, rubbing of nose, &c. The infection, as a rule, lasted from 3 to 5 days, and was usually marked by a racking cough and scanty muco-purulent secretion. Complications developed in from 24–48 hours and were as follows: sinusitis of the antrum of Highmore 5, broncho-pneumonia 2. Autopsies of the two latter showed extensive haemorrhages, peribronchial areas of consolidation with exudate of leucocytes, mononuclear and epithelial cells together with an infiltration and thickening of the alveolar walls. *B. influenzae* was obtained from both lungs in pure culture. In the second series 10 monkeys were inoculated with 1–5 c.cm. of the culture intra-tracheally through the skin by means of a syringe. Of the 10 monkeys thus inoculated 2 developed tracheo-bronchitis and 7 pneumonia. Necropsies of the pneumonia monkeys showed a

widespread fatty pneumonia with extensive haemorrhages, patchy emphysema, oedema, and slight cellular infiltration. In some of the animals the type of pneumonia was rather different, in that it was much more general and there was extensive bronchiolitis. The authors consider that the pathology of the lungs in the animals referred to above is identical with that observed in influenza in man.

A very distinctive feature of these experiments was the extreme prostration which the animals showed. The extreme and often sudden prostration, one of the chief clinical features of acute influenza, is suggestive of some sudden intoxication. Little, however, had been done to confirm this idea until Julia Parker (69), working along the lines which had been followed so successfully in the case of the anaerobes of gas gangrene, demonstrated the existence of a soluble poison in young boiled blood broth cultures. We have found that for rabbits the lethal dose of the filtrates of these cultures varied as a rule from 5 to 6 c.cm. or less. In some cases death followed almost immediately, in others the animal became ill and lay in a collapsed condition, death usually following next day. In these animals a post-mortem examination revealed numerous subcutaneous haemorrhages and characteristic lung changes.

In the guinea-pig the average intravenous lethal dose is 2 or 3 c.cm. although with some cultures it was as low as 0·5 c.cm.

Perhaps the most suggestive train of symptoms were those produced in guinea-pigs, whose death occurred in four or five hours after the injection. Immediately following the injection the animal becomes collapsed, its hair stands on end, and it tends to crouch in a corner of the cage. About an hour later the temperature is found to be subnormal, there is a discharge of frothy mucus at the nose and slight diarrhoea. The pathological changes observed were often quite striking, in fact one is surprised that such extensive changes could have occurred in such a relatively short period of time. This is particularly true of those animals which die in from 24 to 48 hours. The changes are identical with those noted in the case of rabbits, only they are more intense. The lungs are as a rule voluminous and do not retract fully when the chest is opened. On the surface is to be seen slightly sunken red areas alternating with paler lung tissue. In some definite haemorrhagic areas are observed; these at times may involve a whole lobe of the lung. On cutting into the lungs blood-stained frothy mucus escapes in considerable amount. In addition these animals have an increased susceptibility to other bacteria (pneumococci, streptococci, &c.).

Microscopically the affected areas show a typical picture of broncho-pneumonia with proliferation and desquamation of the alveolar and bronchial mucous membrane, and an infiltration of leucocytes, while the bronchioles are plugged with desquamated epithelium, leucocytes and mucus. In some the lesion was distinctly haemorrhagic in character. In most there was a considerable effusion of albuminous fluid in the alveoli of the lungs.

The supra-renals are oedematous and frequently the seat of numerous haemorrhages. The kidneys show a varying amount of parenchymatous degeneration with actual necrosis of the epithe-

lium in places. The intestines are usually congested and distended with gas, and in places show subperitoneal haemorrhages.

These experimental lesions produced by the soluble toxin of *B. influenzae* cultures present a very striking resemblance to those met with in fatal cases of influenza. The exact nature of this poison is still under observation. The important point, however, is that neither the filtrates of pneumococci nor streptococci produce these extensive lung changes. (McIntosh, 70.)

Immunity.

The presence of specific immune bodies (agglutinins, precipitins, complement fixors) in the blood serum of convalescent influenza patients as evidence of an infection by *B. influenzae* has already been commented on. As further evidence of the parasitic nature of the bacillus the effects of vaccination by means of cultures of *B. influenzae* may be quoted. It might be assumed that if it is the cause of epidemic influenza then vaccination by means of the culture should give a considerable degree of protection against the disease. But it should be borne in mind that in the case of *B. influenzae* the problem is not so simple as with other infections in which the bacteria invade the tissues. It is quite probable that even with a high humoral anti-influenzal immunity the bacillus may still be able to proliferate in the mucous membrane. In fact, we have had one or two good instances of this where an individual has been vaccinated, and his serum agglutinated *B. influenzae* to a comparatively high dilution, and he has yet developed an acute catarrh, the discharge of which contained the bacillus in large numbers. Under these conditions the attacks are short and seldom last more than 24 hours, the patient feeling none of the usual influenzal prostration.

If influenza be due to an intoxication by the poison of *B. influenzae* rather than to an actual invasion of the tissues, the vaccine, to get the best results, should be made from young cultures of toxin producing strains. In this way a certain amount of active immunization against the toxin may be produced. The best protective results have been obtained by the use of such vaccines. Duval and Harris (71) have shown that about 30 per cent. of individuals inoculated with good vaccines really developed a mild influenzal attack in which all the usual symptoms of influenza were present. We have seen one or two such instances ourselves, and this is especially liable to happen if the patient is vaccinated during the incubation period. These attacks are evanescent and only of a few hours' duration.

As regards figures, perhaps the most suggestive are those obtained by Leishman (72) from Army returns.

		Rate per 1000.		
		Incidence.	*Pulmonary complications.*	*Deaths.*
Inoculated	15,624	14·1	1·6	0·12
Non-inoculated	43,520	47·3	13·3	2·25

Equally striking are those of Duval and Harris who vaccinated over 3,000 individuals with the following result:

	No. vaccinated.	*Developed influenza.*		*Incidence of disease in*	
		No.	*Per cent.*	*vaccinated*	*unvaccinated.*
One injection	118	29	24		
Two injections	346	28	8		
Three ,,	2608	45	1·7	3·3 %	41·6 %
Controls	866	375	41·6		

Eyre and Lowe (31) among New Zealand troops obtained the following figures:

	Inoculated.	*Uninoculated.*
Approx. number	16,104	5,700
Incidence	1·3 per cent.	4·1 per cent.
Mortality	0·26 per cent.	2·2 per cent.

Friend (74) reports an apparent immunity among 633 boys at a public school although influenza was present among the servants of several of the houses.

A few writers claim to have obtained unsatisfactory results; in this respect may be mentioned Wadsworth (53), and McCoy, Murray, and Teeter (75), and Meyer (76). Wadsworth reports that in a girls' training school of 461 inmates, where protective inoculation against influenza was adopted, 166 cases of influenza among the vaccinated occurred and 37 among the unvaccinated. *B. influenzae* was found on 32 occasions out of 38 among the unvaccinated cases, and on 26 out of 30 among the vaccinated.

9. Summary.

The almost constant presence of *B. influenzae* in the secretions and lesions of influenza during the late epidemic is indicative of an aetiological relationship. In fact, an incidence of over 80 per cent. may be regarded as sufficiently high to fulfil Koch's first postulate, particularly in view of the difficulty of an accurate clinical diagnosis. In addition the bacillus bears a direct relationship to the lesions. The fact that the bacillus is found in other conditions and in normal individuals, at any rate during the epidemic period, does not militate against its aetiological importance, any more than the finding of pneumococci or meningococci in healthy persons detracts from their aetiological importance. That there are several serological strains of *B. influenzae* is not a serious objection as has been urged by Park and other workers. Recent work on the pathogenic anaerobes has shown that such methods of differentiation are hyperspecific, and that strains differing widely in their agglutination reactions were found to be one and the same bacillus when tested by the toxin anti-toxin method. The immune serum of one protects equally well against any of the other strains. The pathogenic nature of *B. influenzae* is further confirmed by the presence of antibodies against it in the blood serum of convalescent influenza patients.

The second postulate which concerns the vitality of the virus is fulfilled by the facility with which *B. influenzae* can be cultivated on the new selective media with great ease.

With regard to the third postulate the position is not quite so clear as the exact relationship of the experimental influenza lesions in animals to those of man is not completely established. The disease produced in animals is only brought about with difficulty, and under conditions which ensure the presence of a large amount of toxin. The bacillus alone is almost ineffective. The disease when produced is an acute inflammation of the respiratory tract with involvement of the lungs, and the lesions observed have a very close resemblance to those of the spontaneous disease in the human subject. If this relationship be accepted then all the criteria by which a bacterium should be judged as the cause of a disease have been fulfilled.

10. Conclusions.

1. The predominating micro-organism in the secretions and lesions of the late epidemic was *B. influenzae* (Pfeiffer), which could be demonstrated in over 80 per cent. of the cases.

2. Other members of the catarrhal group of bacteria (pneumococci, streptococci, Gram-negative bacteria, &c.) were much less frequent.

3. Serological examinations, in particular the complement fixation test, confirmed an infection by *B. influenzae*.

4. Epidemic influenza, as shown by the pathological lesions, is essentially an acute inflammation of the respiratory tract.

5. In the early stages the lung changes are more suggestive of a toxic action than bacterial. In the later stages the lung picture may be complicated by other microbic infections.

6. The therapeutic effect of injections of the pooled sera of influenzal convalescents even in severe cases was very encouraging.

7. *B. influenzae* is a pathogenic microbe, producing its main lethal effect by means of a soluble toxin.

8. The experimental inoculation of animals with filtered cultures of *B. influenzae* produced pathological changes which presented a close resemblance to the changes found in man.

9. Injections of *B. influenzae* vaccine can produce in susceptible individuals a typical influenzal attack of short duration.

10. No evidence was obtained in support of the view that influenza is due to a filter-passing virus.

In conclusion I wish to express my indebtedness to those members of the staff of the London Hospital who permitted me to make use of the clinical material, in particular to Dr. R. A. Rowlands and to Professor Turnbull for the pathological material. Further, I am indebted to the Medical Research Council for the means to carry out this research.

London, *June, 1920.*

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12. Description of Plates.

Fig. I. Rabbit—haemorrhagic broncho-pneumonia produced by an injection of *B. influenzae* toxin.
Weigert's iron-haematoxylin and acid fuchsin.
Obj. $\frac{2}{3}''$, Oc. Zeiss 2.

Fig. II. Rabbit, same as in Fig. I.
Obj. 3 mm., Oc. 4 Zeiss.

Fig. III. Guinea-pig. Section showing plugging of a bronchiole with epithelial débris and proliferation of the epithelium.
Haematoxylin and Eosin.
Obj. $\frac{1}{6}''$. Oc. 4 Zeiss.

Fig. IV. Guinea-pig. Kidney showing haemorrhages and cloudy swelling after an injection of *B. influenzae* toxin.
Weigert's iron-haematoxylin and acid fuchsin.
Obj. $\frac{2}{3}''$, Oc. 2 Zeiss.

Fig. V. Section of rabbit's ear showing haemorrhages and areas of small celled infiltration after intravenous injection of *B. influenzae* toxin.
Weigert's iron-haematoxylin and acid fuchsin.
Obj. $\frac{2}{3}''$. Oc. 4 Zeiss.

Fig. VI. Section of human bronchus of case of fulminating influenza. Note the oedema of the submucous layer and cellular infiltration—compare Fig. V.
Weigert's haematoxylin and acid fuchsin.
Obj. $\frac{2}{3}''$ and Oc. 4 Zeiss.

Fig. VII. Section of lymph channel in neighbourhood of bronchial gland from fatal influenza case. Note numerous *B. influenzae*, a single pneumococcus, and numerous inflammatory cells with dilated channel.
Gram and neutral red.
Obj. $\frac{1}{12}''$ and Oc. 4 Zeiss.

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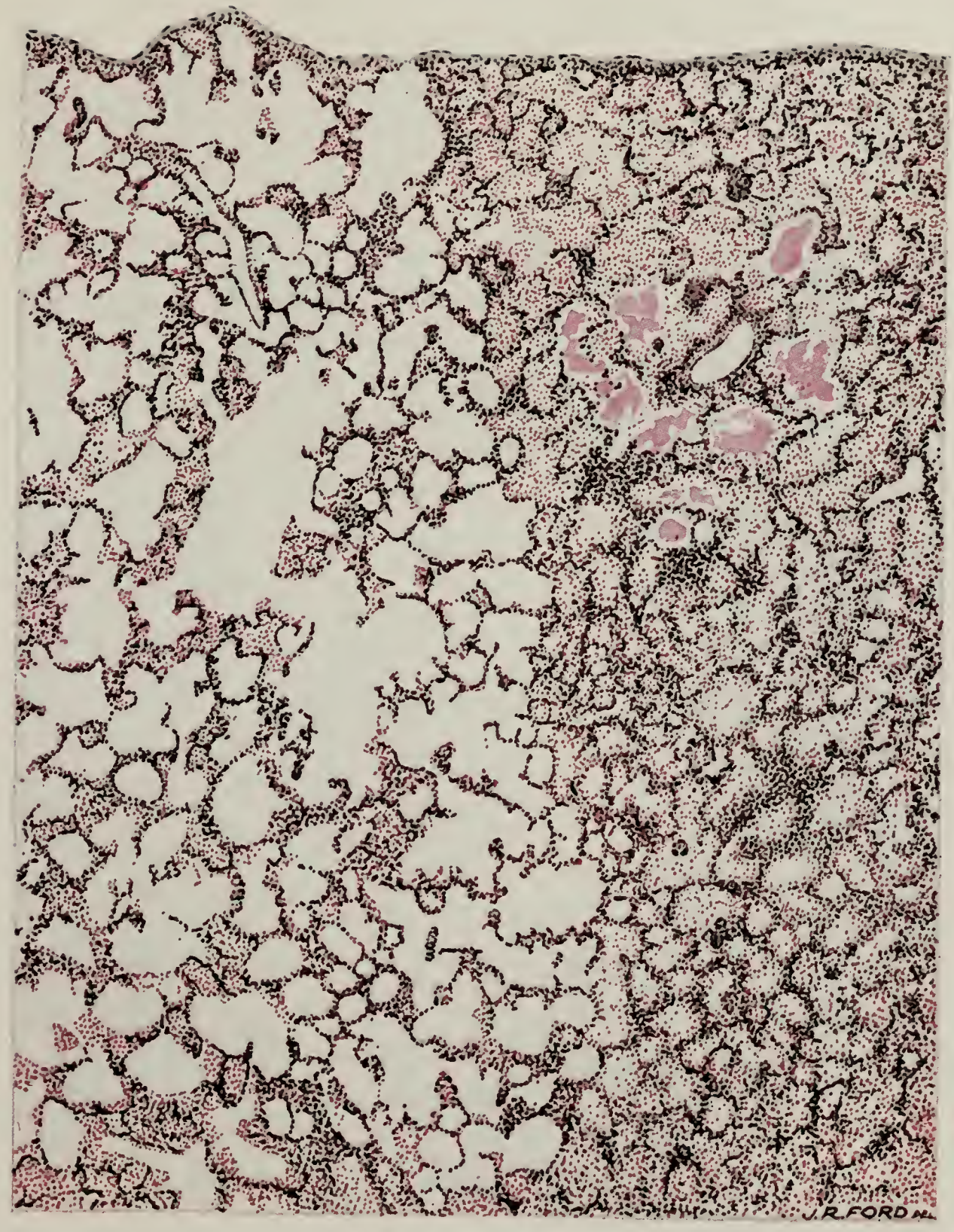

Fig. I

Fig. II

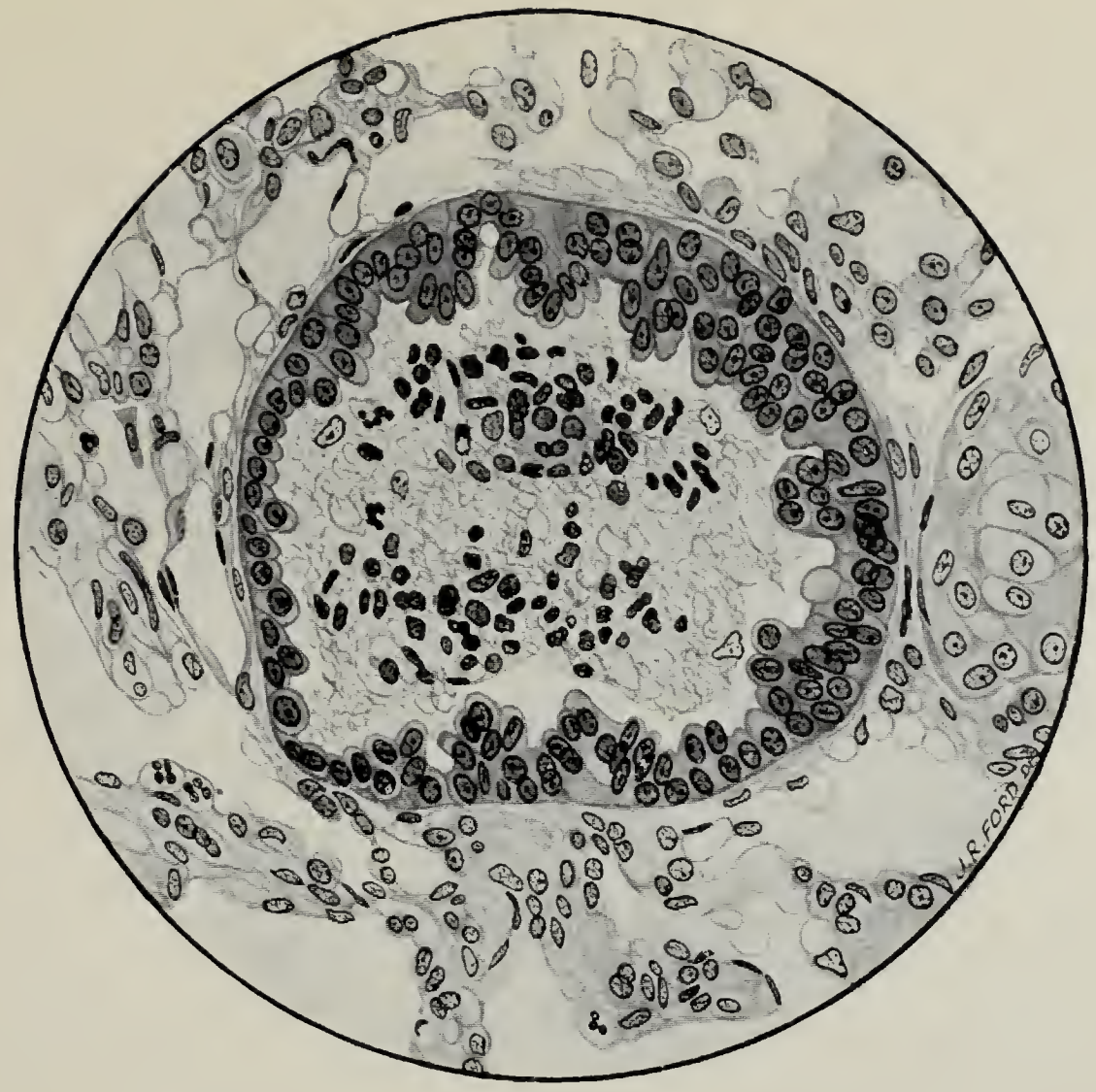

Fig. III

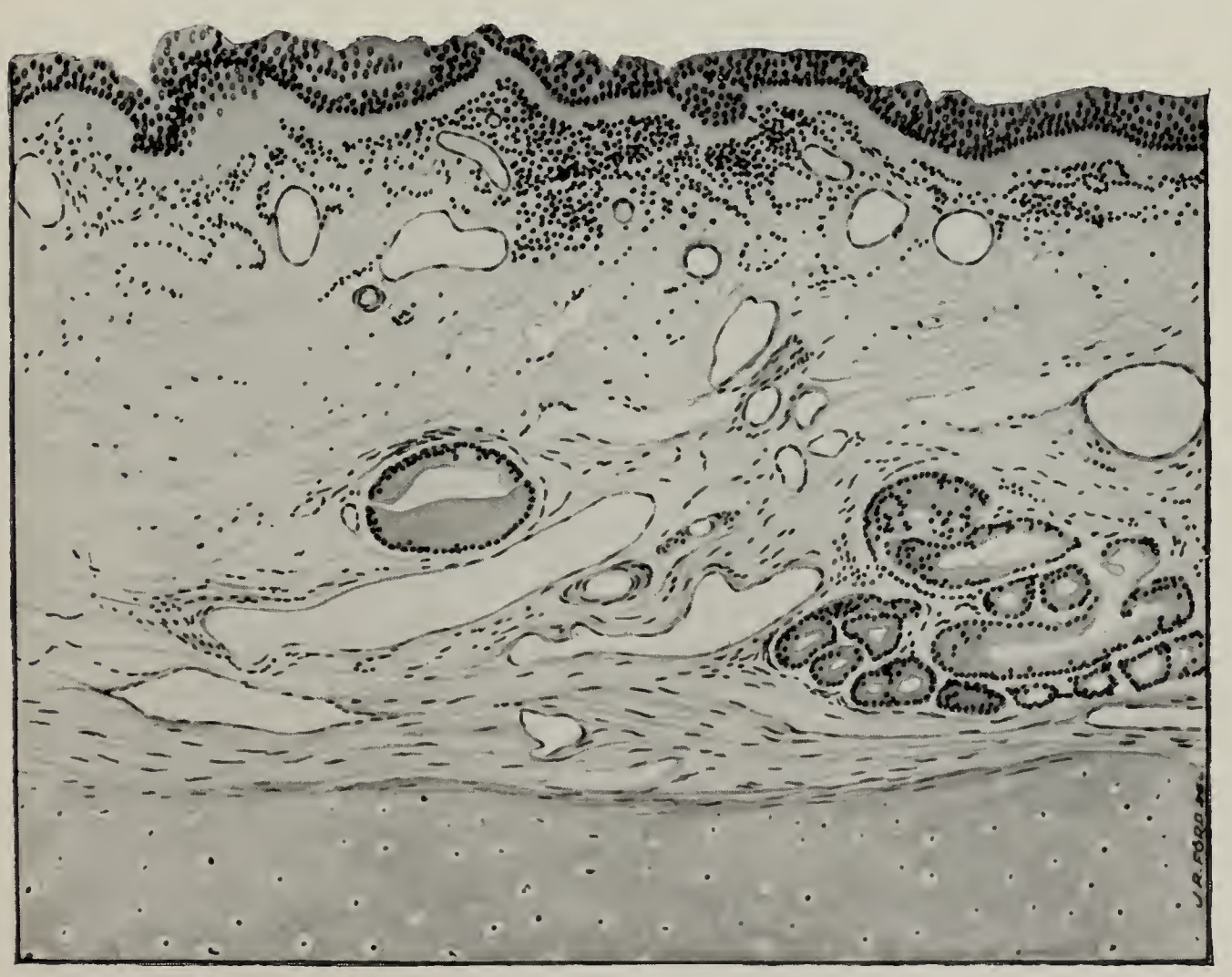

Fig. VI

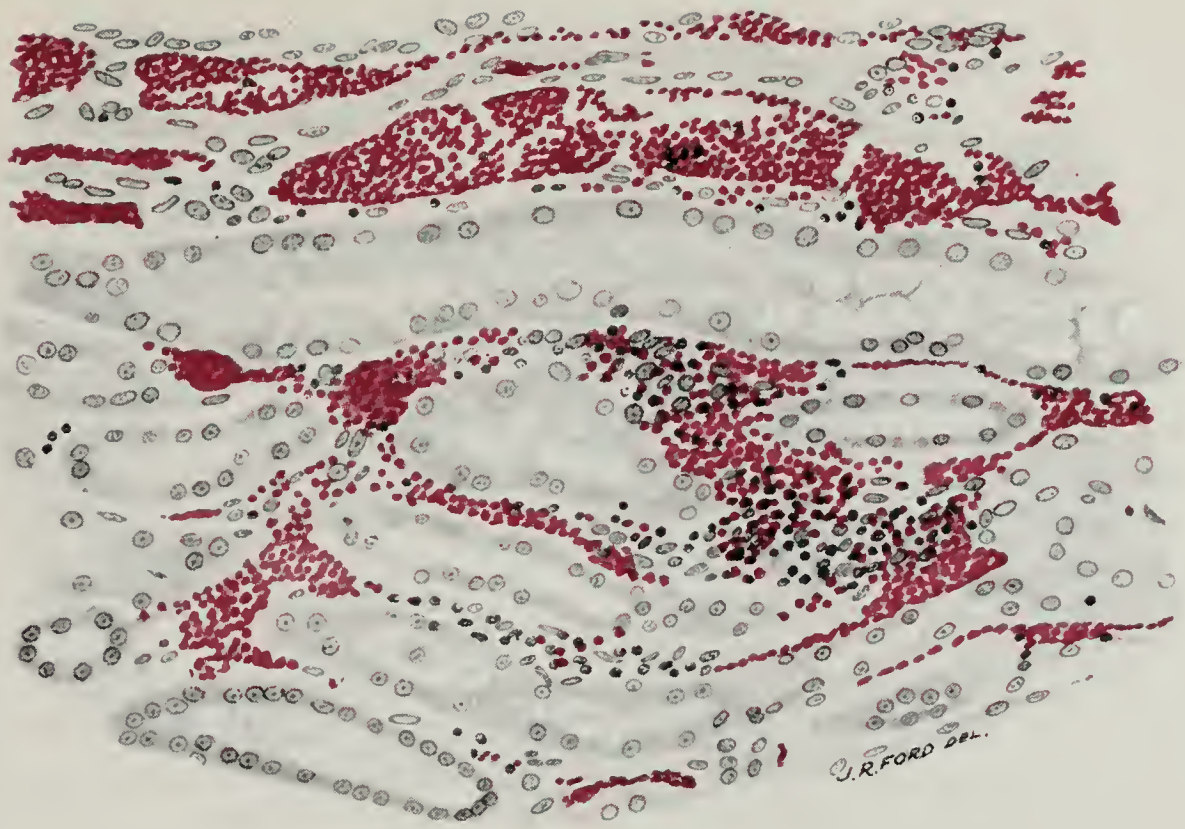

Fig.IV.

Fig.V.

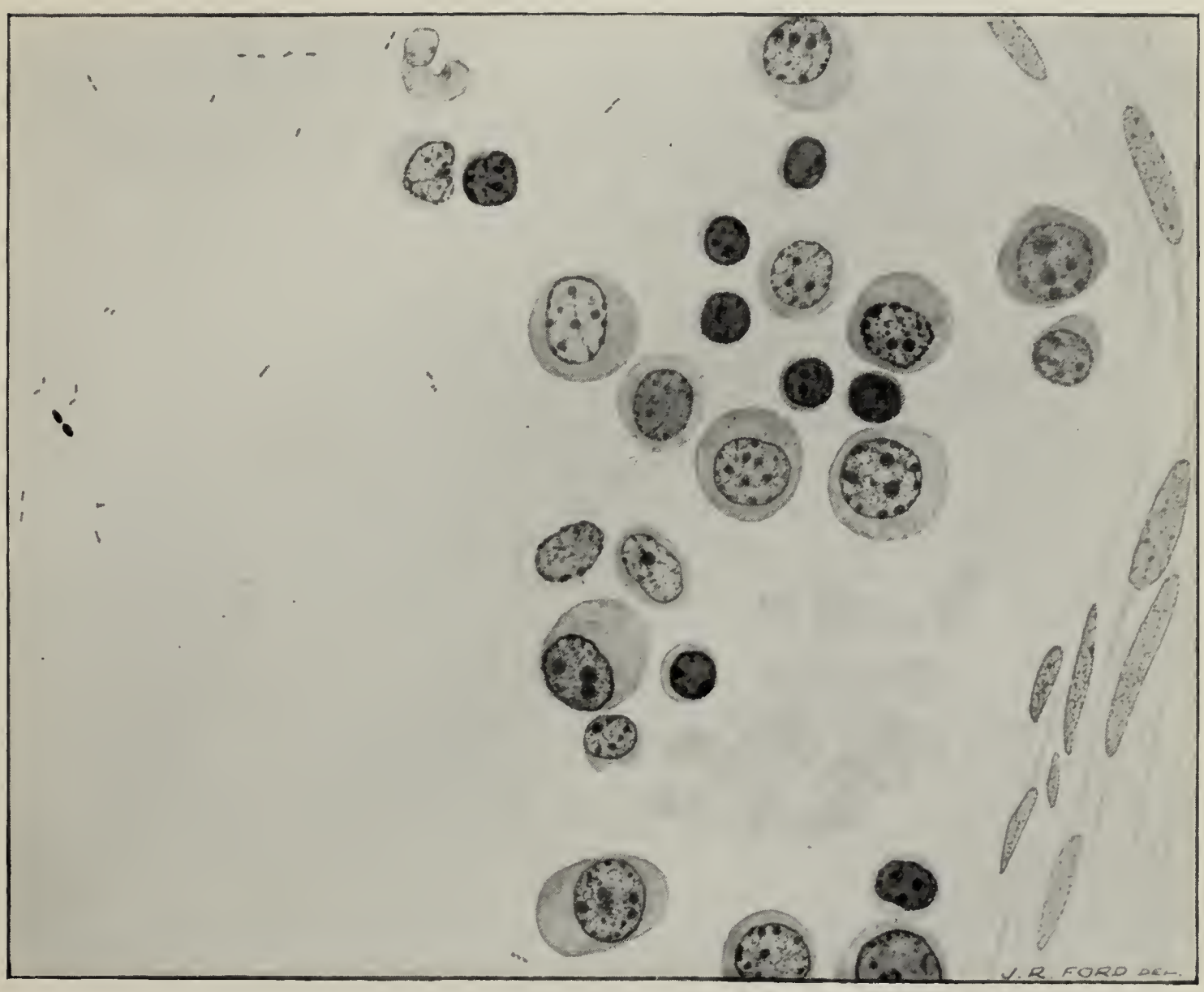

FIG. VII

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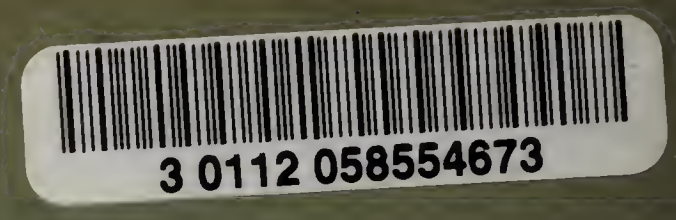
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